

QD  
142  
.A5  
1946

STANDARD METHODS  
FOR THE EXAMINATION OF  
WATER AND SEWAGE

*NINTH EDITION*

1946





LIBRARY

Brigham Young University

GIFT OF

Claude Brown









QD  
142  
.A5  
1946

# STANDARD METHODS FOR THE EXAMINATION OF WATER AND SEWAGE

Prepared, Approved, and Published Jointly by  
the American Public Health Association and  
the American Water Works Association

NINTH EDITION

*Publication Office*

AMERICAN PUBLIC HEALTH ASSOCIATION

1790 Broadway, New York 19, N. Y.

1946

HAROLD B. LEE LIBRARY  
BRIGHAM YOUNG UNIVERSITY  
PROVO, UTAH

NINTH EDITION

First Printing—December, 1946

*Copyright, 1917, 1920, 1923, 1925*

*By the American Public Health Association*

*Copyright, 1933, 1936, 1946*

*By the American Public Health Association  
and the American Water Works Association*

PRINTED IN UNITED STATES OF AMERICA BY  
LANCASTER PRESS, INC., LANCASTER, PA.

**HAROLD B. LEE LIBRARY  
BRIGHAM YOUNG UNIVERSITY  
PROVO, UTAH**



## PREFACE TO THE NINTH EDITION

A preface does not need to be long. It is unnecessary to list all the changes and additions made because the laboratory worker will quickly discover these in the text, and will approve or protest. The Joint Editorial Committee has prepared this Ninth Edition under the stress of the past few years, and it will have to stand on its merits. No manual of laboratory procedures for water and sewage examination is static, nor can it endure more than a few years; neither can it meet the requirements of all water analysts in all interrelated fields. It has been necessary to choose as "standards" those methods which have been most generally accepted and those to which published references can be given. It is hoped that this edition will be received with favor by those especially interested in the various phases of water and sewage analysis.

The greatest value of a foreword to a manual of this character is that it gives an opportunity to acknowledge the assistance rendered by a very large number of interested persons without whose help any attempt to compile standard laboratory procedures would have failed. Appended is a list of committees that have served as active participants in preparing the manuscript, or have assumed special responsibilities. These represent the American Public Health Association, the American Water Works Association, the Federation of Sewage Works Associations, and the American Chemical Society. The Joint Editorial Committee is indebted to all of them.

Special mention should be made of the Standard Methods Committee of the Federation of Sewage Works Associations who compiled the methods in Part II; the Committee on Control of Chlorination and the Committee on Methods of Determining Fluorides of the American Water Works Association; Mac H. McCrady who was largely responsible for the revision of the bacteriologic methods; the late R. D. Scott for helpful criticism of the procedures for determining phenols; and the Secretaries of the two associations who jointly publish this manual, H. E.

Jordan of the American Water Works Association, and R. M. Atwater of the American Public Health Association.

As the manuscript for this Ninth Edition neared completion, it became evident that a more consistent manual could be presented if the final editorial work was concentrated under an experienced editor. Dr. George E. Symons, Associate Editor of "Water and Sewage Works," consented to undertake this work. The Joint Editorial Committee is appreciative of the work he has done and his fine coöperation.

JOHN F. NORTON,  
*Chairman, Joint Editorial Committee*

KENNETH F. MAXCY,  
*for the American Public Health Association*

MALCOLM PIRNIE,  
*for the American Water Works Association*



## JOINT EDITORIAL COMMITTEE

A. M. BUSWELL    W. L. MALLMANN    JOHN F. NORTON  
*for the American Public Health Association*

W. D. HATFIELD    H. A. LEVERIN    M. C. SCHWARTZ \*  
*for the American Water Works Association*

*Editor:* GEORGE E. SYMONS

## LIST OF COMMITTEES COÖPERATING IN THE PREPARATION OF THE NINTH EDITION

### *Committee on Research and Standards, A. P. H. A.*

KENNETH F. MAXCY, *Chairman*; G. W. ANDERSON, J. J. BLOOMFIELD, A. L. BURGDOFF, H. L. DUNN, HAVEN EMERSON, G. M. FAIR, THOMAS FRANCIS, JR., JAMES GIBBARD, LUCY S. HEATHMAN, JOHN F. NORTON, E. S. ROBINSON, T. F. SELLERS, F. W. TANNER, W. D. TIEDEMAN, R. M. ATWATER, *Secretary*; W. L. HALVERSON, *Ex-Officio*; A. P. HITCHENS, *Ex-Officio*.

### *Committee on Water Works Practice, A. W. W. A.*

MALCOLM PIRNIE, *Chairman*; REEVES NEWSOM, FRANK A. BARBOUR, CHARLES R. COX, W. W. DEBERARD, ROSS L. DOBBIN, EUGENE F. DUGGER, ARTHUR E. GORMAN, LOUIS R. HOWSON, WM. W. HURLBUT, R. C. KENNEDY, A. P. KURANZ, WENDELL R. LADUE, DALE L. MAFFITT, HUBERT F. O'BRIEN, WILLIAM STORRIE, N. T. VEATCH, W. H. WEIR, W. VICTOR WEIR, ABEL WOLMAN.

### *Committee on Methods for the Examination of Water and Sewage, A. C. S.*

A. M. BUSWELL, *Chairman*; W. D. COLLINS, F. W. MOHLMAN, F. G. STRAUB.

### *Coördinating Committee on Standard Methods, Laboratory Section, A. P. H. A.*

A. P. HITCHENS, *Chairman*; R. S. BREED, THOMAS FRANCIS, JR., JAMES GIBBARD, R. A. KELSER, W. L. MALLMANN, F. L.

\* Deceased.

MICKLE, STUART MUDD, E. S. ROBINSON, W. D. STOVALL,  
E. K. KLINE, *Ex-Officio*.

*Standard Methods Committee on Examination of Water and  
Sewage, Laboratory Section, A. P. H. A.*

W. L. MALLMANN, *Chairman*; A. M. BUSWELL, F. W. GILCREAS, MAC H. MCCRADY, M. S. NICHOLS, T. A. OLSON, C. A. STUART, J. T. TRIPP.

*Committee on Control of Chlorination, A. W. W. A.*

C. K. CALVERT, *Chairman*; H. A. FABER, DOUGLAS FEBEN, H. H. GERSTEIN, F. W. GILCREAS, A. E. GRIFFIN, H. O. HARTUNG, P. C. LAUX, W. L. MALLMANN, R. D. SCOTT.

*Committee on Methods of Determining Fluorides, A. W. W. A.*

A. P. BLACK, *Chairman*; W. D. COLLINS, RAY F. GOUDEY, PAUL D. HANEY, M. STARR NICHOLS, R. D. SCOTT. *Cooperators*: C. H. CONNELL, H. V. SMITH, J. G. WEART, L. V. WILCOX.

*Standard Methods Committee, F. S. W. A.*

W. D. HATFIELD, *Chairman*; S. E. COBURN, D. E. BLOODGOOD, A. J. CASTRO, G. P. EDWARDS, KEENO FRASCHINO, H. W. GEHM, F. W. GILCREAS, E. F. HURWITZ, A. J. FISCHER, H. HEUKELEKIAN, W. S. MAHLIE, E. W. MOORE, M. S. NICHOLS, RICHARD POMEROY, C. C. RUCHHOFT, WILLEM RUDOLFS, GEORGE E. SYMONS.

*Publications Committee, F. S. W. A.*

F. W. GILCREAS, *Chairman*; W. H. WISELY, F. W. MOHLMAN, ROLF ELIASSEN, CARL E. GREEN, F. M. VEATCH, C. C. LARSON.



# CONTENTS

	PAGE
PREFACE TO THE NINTH EDITION .....	iii
LIST OF COMMITTEES COÖPERATING IN THE PREPARATION OF THE NINTH EDITION .....	v

## PART I

### Examination of Water—Physical and Chemical

INTRODUCTION .....	1
1. COLLECTION OF SAMPLES .....	1
A. Quantity of Water Required for Analysis .....	1
B. Time Interval Between Collection and Analysis .....	1
C. Representative Samples .....	2
2. LABORATORY APPARATUS .....	4
A. Distilled Water .....	4
B. Bottles .....	4
C. Nessler Tubes .....	4
D. Reagents .....	5
E. Volumetric Glassware .....	5
F. Colorimetric and Photometric Apparatus .....	5
G. Specialized Laboratory Apparatus .....	5
3. EXPRESSION OF CHEMICAL RESULTS .....	5
A. Units .....	5
B. Hypothetical Combinations .....	7
C. Classification of Results .....	9
4. DETERMINATIONS .....	9
5. TEMPERATURE .....	10
6. TURBIDITY .....	10
A. Turbidity Measurements—Standard .....	10
B. Turbidity Measurements—Above 100 .....	12
C. Turbidity Measurements—Between 5 and 100 .....	12
D. Turbidity Measurements—Less than 5 .....	13
E. Recording Turbidity Readings .....	14
7. COLOR .....	14
A. Comparison with Platinum-Cobalt Standards .....	14
B. Comparison with Glass Discs .....	15
8. ODOR .....	16
9. RESIDUE .....	20
A. Residue on Evaporation .....	20
B. Residue by Electrolytic Conductivity—Total Dissolved Solids .....	21
C. Residue by Calculation—Total Dissolved Solids .....	22

	PAGE
10. HARDNESS.....	23
A. Total Hardness by Calculation.....	23
B. Hardness by Palmitate—Total, Magnesium, and Calcium..	23
C. Hardness by Soap—Total, Calcium, Magnesium, and Low Hardness (Zeolite Effluent).....	25
D. Hardness by Soda Reagent.....	27
11. pH VALUE.....	28
A. Electrometric—Glass Electrode.....	28
B. Colorimetric.....	30
12. ACIDITY.....	31
A. Volumetric.....	31
13. ALKALINITY.....	31
A. Volumetric.....	31
14. CARBON DIOXIDE.....	32
A. Total Carbon Dioxide by Evolution Method.....	32
B. Free or Uncombined Carbon Dioxide by Calculation.....	34
C. Free or Uncombined Carbon Dioxide by Titration.....	34
15. BICARBONATE ION.....	37
A. Bicarbonate from Total Carbon Dioxide by Calculation...	37
B. Bicarbonate from Alkalinity by Calculation.....	37
16. CARBONATE ION.....	38
A. Carbonate from Total Carbon Dioxide by Calculation.....	38
B. Barium Chloride Method.....	39
C. Carbonate from Alkalinity by Calculation.....	39
17. HYDROXIDE.....	40
A. Strontium Chloride Method.....	40
B. Barium Chloride Method.....	41
C. Hydroxide from Alkalinity by Calculation.....	42
18. OIL.....	42
A. Evaporation and Extraction.....	42
B. Wet Extraction Method.....	43
19. SILICA.....	43
A. Gravimetric.....	43
B. Colorimetric Method—Molybdate.....	44
C. Modification—For Testing Condensate. Molybdate and Reduction with Amino Naphthol Sulfonic Acid.....	45
D. Colorimetric Method—Molybdate and Reduction with So- dium Sulfite.....	46
20. ARSENIC.....	46
21. COPPER.....	47
A. Colorimetric—Carbamate.....	47
22. LEAD.....	49
23. ALUMINUM.....	50
A. Gravimetric.....	50
B. Colorimetric.....	50
24. IRON.....	51
A. Gravimetric.....	51



# CONTENTS

ix

	PAGE
B. Colorimetric—Bipyridine Method.....	51
C. Colorimetric—Phenanthroline Method.....	52
D. Colorimetric—Thiocyanate Method for Total Iron.....	53
25. CHROMIUM.....	55
A. Colorimetric.....	55
26. MANGANESE.....	56
A. Colorimetric—Periodate Method.....	56
B. Colorimetric—Persulfate Method.....	57
27. ZINC.....	58
A. Nephelometric.....	58
28. CALCIUM.....	59
A. Gravimetric.....	59
B. Volumetric.....	60
29. MAGNESIUM.....	60
A. Gravimetric.....	60
B. Colorimetric.....	61
30. SODIUM.....	62
A. Gravimetric.....	62
31. POTASSIUM.....	62
A. Gravimetric—Perchlorate Method.....	62
B. Colorimetric.....	64
32. AMMONIA NITROGEN.....	64
A. Distillation.....	65
B. Preparation of Permanent Standards.....	67
C. Direct Nesslerization.....	67
33. ALBUMINOID NITROGEN.....	68
34. ORGANIC NITROGEN.....	68
35. NITRATE NITROGEN.....	69
A. Phenoldisulfonic Acid Method.....	69
B. Reduction Method.....	70
36. NITRITE NITROGEN.....	71
37. CHLORIDE.....	73
A. Volhard Method.....	73
B. Mohr Method.....	73
38. IODIDE.....	74
39. FLUORIDE.....	76
A. Without Distillation.....	76
B. With Distillation.....	77
40. ORTHOPHOSPHATE.....	79
A. Gravimetric.....	79
B. Colorimetric—Amino-Naphthol-Sulfonic Acid Method.....	79
C. Colorimetric—Stannous Chloride Method.....	80
41. PYROPHOSPHATE.....	81
A. Manganous Chloride Separation Method.....	81
B. Difference Method.....	82
42. METAPHOSPHATE.....	82
A. Barium Chloride Separation Method.....	82
B. Difference Method.....	83

	PAGE
43. SULFATE.....	83
A. Gravimetric.....	83
B. Benzidine Method.....	84
C. Tetrahydroxyquinone Method.....	85
44. SULFITE.....	86
A. Volumetric.....	86
45. SULFIDES.....	87
A. Sampling.....	87
B. Total Sulfides by Evolution Method.....	87
C. Dissolved Sulfides by Evolution Method.....	87
D. Dissolved Sulfides by Volumetric Method.....	87
E. Total and Dissolved Sulfides by Colorimetric Method.....	87
46. BORON.....	87
47. CYANIDE.....	90
A. Colorimetric.....	90
48. TANNIN AND LIGNIN.....	91
49. RESIDUAL CHLORINE.....	92
A. Ortho-tolidine Method.....	93
B. Iodometric Method.....	98
C. Ortho-tolidine Flash Test Method.....	100
D. Ortho-tolidine-Arsenite (OTA) Method.....	100
E. Drop Dilution Method for Field Use.....	102
50. CHLORINE DEMAND.....	103
A. Method for Laboratory Use.....	103
B. Method for Field Use.....	104
51. DISSOLVED OXYGEN.....	106
52. HYDROGEN SULFIDE.....	110
A. Sampling.....	110
B. Free or Uncombined Hydrogen Sulfide by Calculation.....	111
53. METHANE.....	111

## PART II

### Sewage, Sewage Effluents, Industrial Wastes, Polluted Waters, Sludges, and Muds

1. COLLECTION OF SAMPLES.....	113
A. Representative Samples.....	113
B. Method of Sampling Sewage and Effluents.....	113
C. Preservation of Samples.....	113
2. EXPRESSION OF CHEMICAL RESULTS.....	114
3. PHYSICAL EXAMINATION.....	115
A. Temperature.....	115
B. Turbidity.....	115
C. Color.....	115
D. Odor.....	115



# CONTENTS

xi

	PAGE
4. AMMONIA NITROGEN.....	115
A. Direct Nesslerization.....	116
B. Preparation of Permanent Standards.....	117
C. Distillation Method.....	117
5. ORGANIC NITROGEN.....	118
6. TOTAL KJELDAHL NITROGEN.....	119
7. NITRATE NITROGEN.....	119
A. Reduction Method.....	119
B. Phenoldisulfonic Acid Method.....	120
8. NITRITE NITROGEN.....	121
9. OXYGEN CONSUMED FROM PERMANGANATE.....	122
10. DISSOLVED OXYGEN.....	124
A. Collection of Samples.....	124
B. Selection of a Method of Procedure.....	126
C. Standardization of Thiosulfate Solutions.....	126
D. The Winkler Method.....	127
E. The Alsterberg or Sodium Azide Modification.....	129
F. The Rideal-Stewart or Permanganate Modification.....	130
G. The Alkaline-Hypochlorite Modification.....	132
H. The Alum Flocculation Modified Winkler Procedure.....	134
I. Dissolved Oxygen in Activated Sludge Mixed Liquor.....	134
J. Outline for Dissolved Oxygen Procedure.....	135
11. BIOCHEMICAL OXYGEN DEMAND.....	139
A. Dilution Method for Sewage.....	139
B. Dilution Method for Sludges and Muds.....	143
12. RELATIVE STABILITY OF EFFLUENTS.....	144
13. RESIDUE OR SOLIDS.....	145
A. Total Solids on Evaporation.....	145
B. Suspended Solids.....	145
C. Dissolved Solids.....	146
D. Settleable Solids.....	146
14. ACIDITY.....	146
15. ALKALINITY.....	147
16. PH VALUE.....	147
17. CHLORIDE.....	147
18. RESIDUAL CHLORINE.....	147
A. Ortho-tolidine Method.....	147
B. Starch-Iodide Method.....	148
C. Spot Plate Test for Field Use.....	150
19. CHLORINE DEMAND.....	150
20. SULFIDES (TOTAL, DISSOLVED, AND H <sub>2</sub> S).....	152
A. Sampling.....	152
B. Titration Method.....	152
C. Colorimetric Method (Methylene Blue).....	154
21. GREASE.....	155
22. COLLECTION OF SLUDGE AND MUD SAMPLES.....	156
23. PHYSICAL TESTS ON SLUDGE.....	157

	PAGE
24. SPECIFIC GRAVITY OF SLUDGE.....	157
25. SUSPENDED SOLIDS OF SLUDGES AND AERATION TANK LIQUOR—ALUMINUM DISH METHOD.....	157
26. SETTLEABILITY OF ACTIVATED SLUDGE.....	157
27. SLUDGE VOLUME INDEX (S.V.I.).....	158
28. SLUDGE DENSITY INDEX (S.D.I.).....	158
29. REACTION (ACIDITY, ALKALINITY AND PH) OF SLUDGES.....	158
A. Acidity to Phenolphthalein.....	158
B. Alkalinity.....	158
C. pH Value.....	159
30. MOISTURE AND SOLIDS IN SLUDGE.....	159
A. Moisture and Total Solids.....	159
B. Volatile Solids.....	159
31. NITROGEN IN SLUDGE.....	159
A. Total Nitrogen.....	159
B. Ammonia Nitrogen.....	160
C. Organic Nitrogen.....	160
32. GREASE IN LIQUID SLUDGE.....	160

### PART III

#### Microscopical Examination of Water, Sewage Sludge, and Bottom Sediments

1. DEFINITION AND SCOPE OF WATER EXAMINATIONS.....	162
2. APPARATUS FOR WATER EXAMINATION.....	162
A. Devices for Collecting Samples.....	162
B. Devices for Concentration of Samples.....	163
C. Apparatus for Examination of Water Samples.....	164
3. COLLECTION OF WATER SAMPLES.....	166
4. CONCENTRATION OF WATER SAMPLES.....	167
5. EXAMINATION OF WATER SAMPLES.....	169
A. Calibration of Microscope.....	169
B. Selection of Aliquot Portion.....	170
C. Examination and Enumeration of Organisms.....	170
6. REPORTING RESULTS IN WATER EXAMINATIONS.....	173
A. Forms.....	173
B. Observations and Reporting.....	173
C. Calculation of Results.....	174
7. SCOPE OF EXAMINATION OF SEWAGE SLUDGE AND BOTTOM SEDIMENTS.....	177
8. APPARATUS FOR SLUDGE EXAMINATION.....	177
A. Quantitative Samples.....	177
B. Qualitative Samples.....	177
C. Alternative Apparatus.....	177
9. COLLECTION OF SLUDGE OR SEDIMENT SAMPLES.....	178
10. CONCENTRATION OF SLUDGE OR SEDIMENT SAMPLES.....	178
11. SLUDGE OR SEDIMENT.....	179
A. Examination and Enumeration of Organisms.....	179
12. RECORDING RESULTS OF SLUDGE EXAMINATION.....	180



## PART IV

## Bacteriological Examination of Water —

	PAGE
1. LABORATORY APPARATUS.....	183
A. Sample Bottles.....	183
B. Pipettes.....	183
C. Dilution Bottles.....	183
D. Petri Dishes.....	183
E. Fermentation Tubes.....	183
2. MATERIALS.....	184
A. Water.....	184
B. Meat Extract.....	184
C. Peptone.....	184
D. Sugars.....	184
E. Agar.....	184
F. Gelatin.....	184
G. General Chemicals.....	184
H. Dyes.....	184
3. PREPARATION OF CULTURE MEDIA.....	184
A. Adjustment of Reaction.....	184
B. Sterilization.....	185
C. Clarification.....	185
D. Nutrient Broth.....	185
E. Lactose Broth.....	186
F. Nutrient Gelatin.....	186
G. Nutrient Agar.....	186
H. Tryptone Glucose Extract Agar.....	186
I. Endo Medium.....	187
J. Eosin Methylene-Blue Agar.....	187
K. Brilliant-Green Lactose Bile Broth.....	188
L. Formate Ricinoleate Broth.....	188
M. Lauryl Sulfate Tryptose Broth.....	188
N. Permissible Variations in Media.....	188
O. Storage of Culture Media.....	189
4. SAMPLES.....	190
A. Collection.....	190
B. Storage and Transportation.....	191
5. DILUTIONS.....	191
6. PLATING.....	191
7. INCUBATION.....	192
8. COUNTING.....	192
9. TESTS FOR THE PRESENCE OF MEMBERS OF THE COLIFORM GROUP... 193	193
A. Introduction and Definitions.....	193
B. Presumptive Test.....	194
C. Confirmed Test.....	195
D. Completed Test.....	196
E. Technic for the Gram Stain.....	198
F. Selection of Coliform Tests.....	199

	PAGE
10. SCHEMATIC OUTLINE OF PRESUMPTIVE, CONFIRMED, AND COMPLETED TESTS AS DESCRIBED IN SECT. 9.....	200
A. Presumptive Test.....	200
B. Confirmed Test.....	200
C. Completed Test.....	201
11. ESTIMATION OF COLIFORM GROUP DENSITY.....	202
A. Basis and General Considerations.....	202
B. Water of Presumed Drinking Water Quality.....	202
C. Water Subject to USPHS Drinking Water Standards.....	202
D. Water of Other than Drinking Water Quality.....	203
E. Computing and Recording the Most Probable Number.....	203
F. The Coliform Indicated Number.....	205
G. Precision of the Fermentation Tube Test.....	206
12. INTERPRETATION OF COLIFORM GROUP RESULTS.....	207
13. BACTERIOLOGICAL CONTROL OF SWIMMING POOLS AND BATHING PLACES.....	207
A. Preparation of the Sample Bottle.....	207
B. Collection of Samples.....	208
C. Determination of the Bacterial Count.....	208
D. Determination of the Coliform Group.....	208

## APPENDIX I

### Non-Standard Methods

FOREWORD.....	209
1. CHROMIUM IN WATER.....	209
2. SELENIUM IN WATER.....	210
3. pH BUFFERS AND INDICATORS.....	211
A. Buffer Standards.....	211
B. Indicators.....	213
4. LEAD.....	214
5. PHENOLS IN WATER.....	216
6. PHENOL IN POLLUTED STREAMS AND TRADE WASTES.....	218
7. VOLATILE ACIDS IN DIGESTING SLUDGES.....	218
8. OXYGEN DEMAND AND ACTIVITY OF ACTIVATED SLUDGE.....	219
9. DETERMINATION OF GREASE IN SEWAGE SLUDGE.....	221
A. Wet Extraction Method.....	221
B. Extraction of Coagulated Grease.....	223
10. pH OF SEWAGE AND SLUDGE COLORIMETRICALLY.....	224
11. ORGANIC CARBON IN SEWAGE.....	224
12. BROTHS, SELECTIVE, FOR PRIMARY OR PARALLEL PLANTING.....	226
13. SELECTIVE AGAR MEDIA FOR DIFFERENTIATION IN THE COLIFORM GROUP.....	227
14. DIFFERENTIATION—COLIFORM GROUP ORGANISMS.....	228
A. Culture Purification.....	228
B. Differentiation of Members of the Coliform Group.....	229

# CONTENTS

xv

	PAGE
C. Slow or Weak Lactose Fermenting Organisms.....	230
D. Indole Differential Test.....	230
E. Methyl Red Differential Test.....	230
F. Voges-Proskauer Differential Test.....	231
G. Sodium Citrate Differential Test.....	231
H. Eijkman Differential Test.....	231
15. BRITISH PRACTICE—BACTERIOLOGICAL EXAMINATION.....	233
A. The Rationale of the Bacteriological Analysis of Water.....	234
B. Type of Examination.....	236
C. Interpretation of Results.....	237
D. Suggested Classification of Waters.....	241
E. The Presumptive Coliform Count.....	243

## APPENDIX II

### Chemicals and Reagents

1. REAGENT GRADE CHEMICALS.....	246
2. COMMON LABORATORY REAGENTS.....	248



## INDEX OF TABLES

NO.	TITLE	PART	SEC.	PAGE
1.	Conversion Data for Analytical Results .....	I	3	6
2.	Conversion Table for Hardness.....	I	3	6
3.	Factors to Convert Equivalents per Million to Parts Per Million and Vice Versa.....	I	3	7
4.	Relations Between Alkalinity to Phenolphthalein and that to Methyl Orange in Presence of Hydroxide, Carbonate, and Bicarbonate....	I	3	9
5.	Graduation of Candle Turbidimeter.....	I	6	11
6.	Odor Characteristics.....	I	8	19
7.	Indicators, and the Volume of 0.05 N Sodium Hydroxide Necessary to Effect Solution of 0.1 gram.....	I	11	30
8.	Relationships Between pH and Fractional Activities of Alkalinity and Acidity Con- stituents.....	I	14	36
9.	Preparation of Permanent Standards for the De- termination of Iron.....	I	24	55
10.	Color Standards for Determination of Manganese	I	26	57
11.	Preparation of Permanent Standards for the De- termination of Ammonia Nitrogen.....	I	32	67
12.	Chlorine Standards—Modified Scott Formula— 0.01–1.0 ppm.....	I	49	96
13.	Chlorine Standards—Modified Scott Formula— 1.0–10.0 ppm.....	I	49	96
14.	Solubility of Oxygen in Fresh Water and in Sea Water.....	II	10	137
15.	Relative Stability Numbers.....	II	12	144
16.	Hydrogen Sulfide Factors.....	II	20	153
17a-b.	Most Probable Numbers—Coliform Group Tests.....	IV	11	204-5
18.	Composition of Mixtures Giving Various pH Values.....	App. I	3	212
19.	Indicators—pH Ranges from 1.2 to 9.8.....	App. I	3	213
20.	Coliform Group-Reaction Classification.....	App. I	14	229
21.	Differentiation of the Coliform Group.....	App. I	15	238
22.	Reagent List—American Chemical Society.....	App. II	1	246
23.	U. S. Bureau of Standards—Standardization Samples.....	App. II	1	248

## INDEX OF FIGURES

NO.	TITLE	PART	SEC.	PAGE
1.	Cooling Coil for Sampling Boiler Water.....	I	1	2
2.	Cooling Coil for Sampling Boiler Water.....	I	1	3
3.	Apparatus Assembly for Evolution Method for Determining Total Carbon Dioxide.....	I	14	33
4.	Graph Showing Relation of pH and Carbon Di- oxide, Carbonate and Bicarbonate Ions at Different Fractional Activities.....	I	14	35
5.	Fluoride Distillation Assembly.....	I	39	78
6.	Circuit Diagram of the Electrometric Titration Apparatus.....	I	46	88
7.	Dissolved Oxygen in Boiler Water-Sampling Flask.....	I	51	106
8.	New Sampling Assembly for Schwartz and Gurney Oxygen Test.....	I	51	107
9.	Modified Gas Sampling Tubes for Dissolved Oxygen Sampling.....	I	51	108
10.	Sampler for Gasometric Determination of Methane in Water.....	I	53	111
11.	Dissolved Oxygen and Biochemical Oxygen De- mand Sampler Assembly.....	II	10	125
12.	Funnel and Centrifuge Assembly for Concen- trating Organisms.....	III	2	164
13.	Ocular Micrometer Ruling.....	III	2	165
14.	Graph Representing Calibration of Microscope..	III	5	171
15a-b.	Sample Form of Bench Sheet Used with Centrifuge Method.....	III	6	172
16.	Determination of Cubic Standard Units from Linear Dimensions of Organisms.....	III	6	175
17.	Bottom Sampler for Rocky or Pebbly Bottoms...	III	8	178
18.	Sample Form of Bench Sheet Used to Record Organisms in Bottom Sediment Samples....	III	12	181
19.	Schematic Diagram for Apparatus for the De- termination of Oxygen Demand of Activated Sludge.....	App. I	8	220
20.	Apparatus for Determining Organic Carbon in Sewage.....	App. I	11	225





## PART I

### EXAMINATION OF WATER—PHYSICAL AND CHEMICAL

#### Introduction

The methods described in Part I are adapted to the examination of natural and treated waters which are not grossly polluted. A list of examples would include surface water, well water, softened water, circulating water, process water, boiler feed water, and boiler water.

The factors used in this text and the weights of chemicals to be used in preparing volumetric solutions, reagents, and standards are based on the International Atomic Weights of 1943.

Aluminum.....	Al	26.97
Antimony.....	Sb	121.76
Arsenic.....	As	74.91
Barium.....	Ba	137.36
Boron.....	B	10.82
Bromine.....	Br	79.916
Calcium.....	Ca	40.08
Carbon.....	C	12.01
Chlorine.....	Cl	35.457
Chromium.....	Cr	52.01
Cobalt.....	Co	58.94
Copper.....	Cu	63.57
Fluorine.....	F	19.00
Hydrogen.....	H	1.0080
Iodine.....	I	126.92
Iron.....	Fe	55.85
Lead.....	Pb	207.21
Magnesium.....	Mg	24.32
Manganese.....	Mn	54.93
Molybdenum.....	Mo	95.95
Nitrogen.....	N	14.008
Oxygen.....	O	16.000
Phosphorus.....	P	30.98
Platinum.....	Pt	195.23
Potassium.....	K	39.096
Silicon.....	Si	28.06
Silver.....	Ag	107.88
Sodium.....	Na	22.997
Strontium.....	Sr	87.63
Sulfur.....	S	32.06
Zinc.....	Zn	65.38
Zirconium.....	Zr	91.22

#### 1. Collection of Samples

##### A. QUANTITY OF WATER REQUIRED FOR ANALYSIS

A 2 liter sample should be sufficient for an ordinary physical and chemical analysis. In special cases a larger quantity may be required.

##### B. TIME INTERVAL BETWEEN COLLEC- TION AND ANALYSIS

In general, the shorter the time elapsing between collection and analysis of a sample, the more reliable will be the analytical results. Under some conditions, analysis in the field is necessary to secure accurate results because the composition of the water will change before an analysis can be made in the laboratory.

The time that may be allowed to elapse between collection of a sample and the beginning of its analysis cannot be definitely stated. It depends upon the character of the sample, the examinations to be made and other conditions. Changes incident to activities of organisms present may be greatly retarded if samples are kept at a low temperature until they are examined. The following are suggested as fairly reasonable maximum limits:

##### PHYSICAL AND CHEMICAL ANALYSIS

Unpolluted waters .....	72 hours
Fairly pure waters .....	48 hours
Polluted waters .....	12 hours

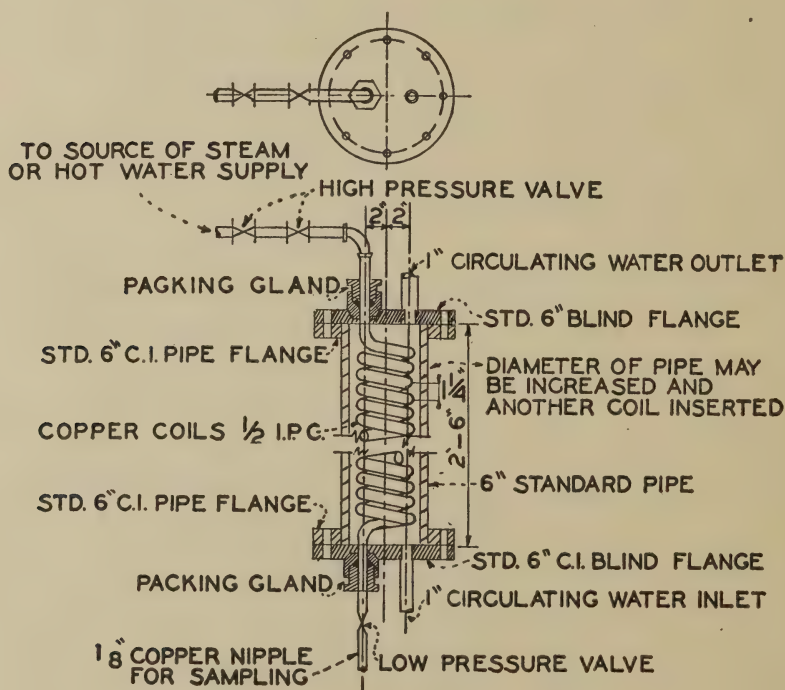
If a longer period elapses between collection and examination the time should be stated. If sterilized by addition of acid or a germicide, samples may be allowed to stand for a longer period than above indicated, but no definite procedure can be recommended.

Determination of dissolved gases, especially oxygen, hydrogen sulfide, and carbon dioxide, should be made at the source in order to be reasonably accurate, in accordance with di-

rections given hereafter in connection with each determination.

### C. REPRESENTATIVE SAMPLES

Care should be taken to obtain a sample that is truly representative of existing conditions and which does not change in composition before analysis. Satisfactory samples of some waters can be obtained only by securing a composite sample collected over a period of time or at different places, the details as to collection de-



**NOTE: DIMENSIONS MAY BE CHANGED TO MEET LOCAL OPERATING CONDITIONS.**

FIG. 1. COOLING COIL FOR SAMPLING BOILER WATER.

FIGS. 1 and 2 do not represent standardized equipment, but illustrate the characteristics of the devices that should be used for cooling samples of boiler water. For more complete data see "Progress Report of Subcommittee No. 8 on Standardization of Water Analyses," *Trans. A. S. M. E.*, 51, 12, 90 (1929).

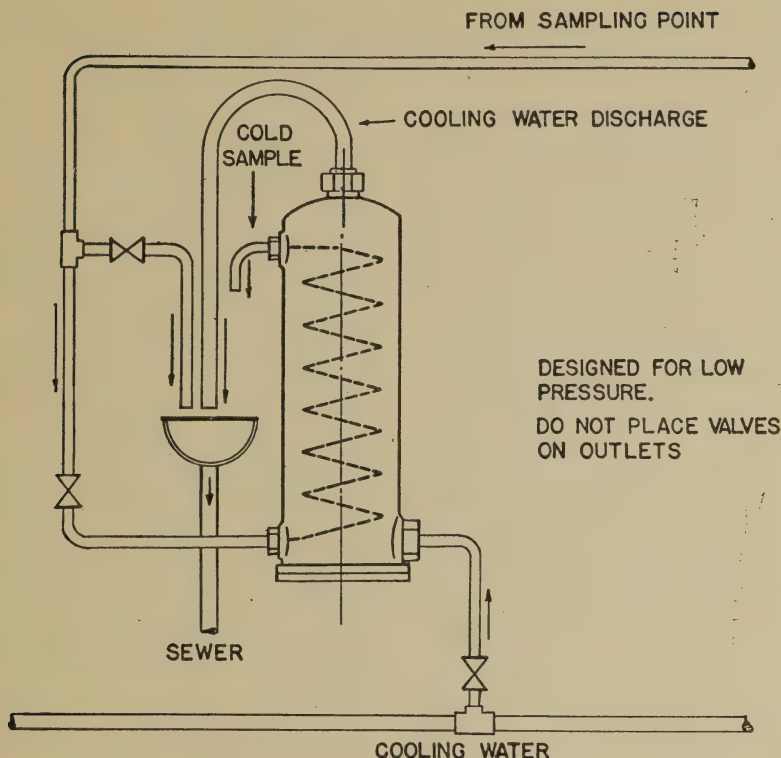


FIG. 2. COOLING COIL FOR SAMPLING BOILER WATER.

pending upon local conditions. In other cases, it will be advisable to collect and analyze samples frequently.

A complete record should be made of information which will positively identify the sample at any later date.

Attention should be paid to the sample containers; a type should be used which will not contaminate the sample. Glass stoppered resistant glass bottles and rubber stoppered rubber bottles are recommended.

In securing samples through metal lines and valves, sufficient flow should be maintained to wash out the system. The sample container should be washed out thoroughly. In certain

cases, the sample will have to be collected out of contact with the atmosphere (i.e., oxygen).

Hot samples under pressure should be cooled down while under pressure to as near 21° C. (70° F.) as possible. A typical installation for collecting such samples is shown in Figs. 1 and 2.

#### BIBLIOGRAPHY

- SPENCER, R. R., AND LETTON, H. P. A new water sample shipping case. *Reprint No. 425, Public Health Repts.*, 1917.
- ELLISON, GAYFREE, HACKLER, H. WATON, AND BUICE, W. ALFRED. Effects of age and storage temperatures on growth of bacteria in water samples. *J. Amer. W. W. Assn.*, 24, 895 (1932).



AMER. SOC. TEST. MTLs. Methods of sampling plant or confined waters for industrial uses. Method D-510-41. *A. S. T. M. Standards, III*, 797 (1942).

## 2. Laboratory Apparatus

### A. DISTILLED WATER

A number of colorimetric methods described in the text are of sufficient sensitivity to respond to impurities in ordinary distilled water. In such cases, double distilled water should be used. The material of which the still is constructed should also be considered in relationship to the use to which the water will be put. Ammonia and carbon dioxide will be present unless special procedures are followed for their removal.

### B. BOTTLES

Glass stoppered resistant glass bottles are recommended in most cases for the collection of samples. For alkaline solutions, rubber stoppered resistant glass bottles or rubber stoppered rubber bottles are recommended.

Sample bottles shall be carefully cleansed each time before using. This may be done by treating with sulfuric acid and potassium dichromate, or with alkaline permanganate, followed by a mixture of oxalic and sulfuric acids and by thorough rinsing with water and draining.

The stoppers and necks of the bottles shall be protected from dirt by tying cloth or thick paper over them.

For shipment, bottles shall be packed in cases with a separate compartment for each bottle. Wooden boxes may be lined with corrugated fiber paper, felt, or similar material,

or provided with spring corner strips, to prevent breakage. Lined wicker baskets also may be used.

### C. NESSLER TUBES

Nessler tubes shall be of the so-called "tall" form. They shall be made of resistant glass from uniformly drawn tubing. The glass shall be clean and colorless. The bottom surfaces shall be parallel planes, and when the tubes are filled with liquid and viewed from the top, using a light source beneath the tube, there shall be no dark spots, and there shall be no distortion of the transmitted light.

The tops of the tubes shall be straight, preferably fire polished and shall be smooth enough to permit the sealing on of protective cover slips when the tubes are filled with color standards.

The graduation marks shall completely encircle the tubes.

One hundred ml. tubes shall have an overall length of approximately 375 mm. The inside diameter of the tubes shall approximate 20 mm. and the outside diameter 24 mm.

The graduation mark on the tube shall be as near 300 mm. from the inside of the bottom as possible. Tubes in sets shall be of such uniformity that the distance between the bottom and the graduation mark of the longest tube shall not exceed that of the shortest tube by more than 6 mm. Sets of these tubes are available in which the difference in height of graduation marks among tubes in a set is not more than 2 mm.

Fifty ml. tubes shall have an overall length of about 300 mm. The inside diameter of the tubes shall ap-

proximate 17 mm. and the outside diameter 21 mm. The graduation mark on the tube shall be as near 225 mm. from the inside of the bottom as possible. Tubes in sets shall be of such uniformity that the distance between the bottom and the graduation mark of the longest tube shall not exceed that of the shortest tube by more than 6 mm. Sets of these tubes are available in which the difference in height of graduation marks among tubes in a set is not more than 1.5 mm.

Tubes for candle turbidimeters shall conform to all the requirements as to quality, color of glass, and workmanship set forth for Nessler tubes. Graduation marks shall conform precisely to the measurements given in the Table in Part I, Sec. 6, page 11.

#### D. REAGENTS

It is assumed throughout the directions following that the reagents used will be the best available and of sufficiently high quality to insure accurate results. This point is not discussed under individual methods.

Reagents should be of no lower grade than that commonly termed *cp.* It is preferable that reagent grade materials be used. Section 1 of Appendix II lists all specifications for reagent chemicals adopted by the American Chemical Society. It also carries reference to the Bureau of Standards certified material for standardization of solutions. Various organic reagents are somewhat unstable on exposure to the atmosphere. When the stability of such reagents is not definitely known, it is advisable to

purchase such materials in small lots.

#### E. VOLUMETRIC GLASSWARE

Volumetric glassware should preferably be calibrated by the analyst using it or by some laboratory which can furnish satisfactory certificates of accuracy.

#### F. COLORIMETRIC AND PHOTOMETRIC APPARATUS

In addition to Nessler tubes, there are available Duboseq type colorimeters, filter photometers, and spectro-photometers for use in colorimetric estimations. Where "permanent" colorimetric standards, either glass or liquid, are used, the analyst should satisfy himself as to their accuracy and check them frequently. For use with Nessler tubes or a Duboseq type colorimeter, a constant light source is advised.

#### G. SPECIALIZED LABORATORY APPARATUS

There is available a variety of laboratory apparatus which will be of value in carrying out the methods described in the text, even though in some cases their use is not mentioned specifically. Examples of such equipment are electronic pH meters, electrolytic conductivity bridges, electrometric titration assemblies. The spectrograph and polarograph have not, as yet, come into general use as tools in the analysis of water.

### 3. Expression of Chemical Results

#### A. UNITS

The results of chemical analyses shall be expressed in terms of sub-

stances actually determined; the values to be reported in parts per million (ppm.). A part per million is actually a weight ratio; it is equivalent to a milligram per liter assuming that a liter of water, measured out for analysis, weighs exactly 1. kilogram. When the error introduced by this assumption exceeds  $\pm 3$  parts in a thousand, the specific gravity of the sample (referred to water at  $4^{\circ}$  C.) shall be determined and results obtained with measured samples shall be divided by this specific gravity to give parts per million. A liter of pure water at  $25^{\circ}$  C. weighs 0.997 kg. in air, and a liter of water containing 6573 mg. of sodium chloride at  $20^{\circ}$  C. weighs 1.003 kg.

Results may also be expressed in

equivalents per million (epm.) which is likewise a weight ratio. The concentration in equivalents per million is calculated by dividing the concentration in parts per million by the chemical combining weight of the substance or ion. If the specific gravity of the solution is exactly 1.0, the term is also equal to milliequivalents per liter. The advantage of expressing results in equivalents per million is the ease with which the positive and negative ions may be grouped and checked against each other. This is particularly useful when hypothetical combinations are being made.

Useful data for the conversion of analytical results are given in Tables 1, 2, and 3.

TABLE 1.—CONVERSION DATA FOR ANALYTICAL RESULTS

.1 epm. = 50 ppm. as $\text{CaCO}_3$
= 2.92 grain per U. S. gallon as $\text{CaCO}_3$
= 3.51 grain per Imperial gallon as $\text{CaCO}_3$
1 ppm. as $\text{CaCO}_3$ = 0.02 epm.
1 ppm. = 0.058 grain per U. S. gallon
1 ppm. = 0.071 grain per Imperial gallon
1 grain per U. S. gal. as $\text{CaCO}_3$ = 0.3424 epm.
1 grain per U. S. gal. = 17.12 ppm.
1 grain per U. S. gal. = 1.20 grain per Imperial gallon
1 grain per Imperial gal. as $\text{CaCO}_3$ = 0.285 epm.
1 grain per Imperial gal. = 14.25 ppm.
1 grain per Imperial gal. = 0.835 grain per U. S. gallon

TABLE 2.—CONVERSION TABLE FOR HARDNESS

Unit	Equivalent				
	Parts per million	Grains U. S. gallon	Clark degrees	French degrees	German degrees
One part per million.....	1.0	0.058	0.07	0.10	0.056
One grain per U. S. gallon.....	17.1	1.00	1.20	1.71	0.958
One Clark degree.....	14.3	0.829	1.00	1.43	0.80
One French degree.....	10.0	0.583	0.70	1.00	0.56
One German degree.....	17.9	1.044	1.24	1.78	1.00



TABLE 3.—FACTORS TO CONVERT EQUIVALENTS PER MILLION TO PARTS PER MILLION AND VICE VERSA

Multiply by to change from

Ion	ppm. to epm.	epm. to ppm.	Ion	ppm. to epm.	epm. to ppm.
Ca <sup>++</sup>	0.04990	20.04	CO <sub>3</sub> <sup>-</sup>	0.03333	30.01
Mg <sup>++</sup>	0.08224	12.16	OH <sup>-</sup>	0.05880	17.01
Ba <sup>++</sup>	0.01456	68.68	H <sub>2</sub> PO <sub>4</sub> <sup>-</sup>	0.01031	97.00
Sr <sup>++</sup>	0.02282	43.82	HPO <sub>4</sub> <sup>-</sup>	0.02084	47.99
Zn <sup>++</sup>	0.03059	32.69	PO <sub>4</sub> <sup>=</sup>	0.03159	31.66
Cu <sup>++</sup>	0.03146	31.79	SO <sub>4</sub> <sup>-</sup>	0.02082	48.03
Pb <sup>++</sup>	0.009652	103.60	HSO <sub>4</sub> <sup>-</sup>	0.01030	97.07
Na <sup>+</sup>	0.04348	23.00	SO <sub>3</sub> <sup>-</sup>	0.02498	40.03
K <sup>+</sup>	0.02558	39.10	HSO <sub>3</sub> <sup>-</sup>	0.01234	81.07
NH <sub>4</sub> <sup>+</sup>	0.05543	18.04	S <sup>-</sup>	0.06238	16.03
H <sup>+</sup>	0.9924	1.008	HS <sup>-</sup>	0.03024	33.07
Fe <sup>++</sup>	0.03581	27.93	Cl <sup>-</sup>	0.02820	35.46
Fe <sup>+++</sup>	0.05371	18.62	Br <sup>-</sup>	0.01251	79.92
Al <sup>+++</sup>	0.1112	8.99	I <sup>-</sup>	0.007879	126.92
Mn <sup>++</sup>	0.03641	27.47	F <sup>-</sup>	0.05263	19.00
Mn <sup>++++</sup>	0.07283	13.73	NO <sub>3</sub> <sup>-</sup>	0.01613	62.01
HCO <sub>3</sub> <sup>-</sup>	0.01639	61.02	NO <sub>2</sub> <sup>-</sup>	0.02174	46.01

## B. HYPOTHETICAL COMBINATIONS

Upon concentration and evaporation of a water solution, solid phases will precipitate from solution. Guesses are frequently made of the combination of ions which will give rise to the precipitating solids. Such combinations of ions are called hypothetical combinations. Actually, they cannot exist in solution because the substances are present in the ionic state. The following schemes of hypothetical combinations are frequently used.

1. Combinations of the positive ions are made with the negative ions in the following order:

Positive ions	Negative ions
Calcium	Carbonate
Magnesium	Hydroxide
Ammonium	Sulfate
Sodium	Chloride
Potassium	Nitrate

In alkaline waters containing large amounts of silica, the silica is consid-

ered to be present as a sodium silicate. Otherwise silica is considered as a separate item. Iron and aluminum oxides are considered as separate items.

2. In the case of alkaline waters, if the total hardness is greater than the carbonate and bicarbonate alkalinity, the amount of hardness equivalent to the alkalinity is called carbonate hardness and the amount of hardness in excess of this is called non-carbonate hardness. When the total hardness is equal to or less than the sum of the carbonate and bicarbonate alkalinity, there is no non-carbonate hardness; the total hardness is all carbonate hardness and the difference between the two is sodium carbonate. In the case of acid waters, all the hardness will be non-carbonate hardness.

If the non-carbonate hardness is in excess of the magnesium, calculate the magnesium to magnesium sulfate.

Calculate the remainder of the non-carbonate hardness to calcium sulfate. The alkalinity is then to be reported as due entirely to calcium carbonate.

If the non-carbonate hardness is less than the magnesium, calculate the non-carbonate hardness to magnesium sulfate and the balance of the magnesium to magnesium carbonate. The magnesium carbonate is subtracted from the value for alkalinity. The difference between the alkalinity and the magnesium carbonate is then reported as calcium carbonate.

If the non-carbonate hardness is negative, make proper allowance in the alkalinity value, for sodium carbonate, calculate the magnesium to magnesium carbonate, making further allowance in the alkalinity value, and the balance of the alkalinity is calcium carbonate.

The difference between the total solids and the sum of magnesium sulfate, magnesium carbonate, calcium sulfate, calcium carbonate, sodium chloride, sodium carbonate, iron and aluminum oxides, and silica, gives the amount of non-incrusting sulfates, nitrates and organic matter. If the above difference is negative, the result indicates calcium and magnesium chlorides or nitrates in which case it will be necessary to make a sulfate determination in order to secure the most probable hypothetical combination.

3. Alkalinity may result from one or more of the following three ions. In an ideal system, containing only the anions hydroxide, carbonate, and bicarbonate, the phenolphthalein and methyl orange alkalinity may be used

to calculate the amounts of each ion. In practice, however, the ions may be calculated more accurately from an equation involving both methyl orange alkalinity and the pH, according to sections 15, 16, and 17, pages 37-42.

Using the methyl orange and phenolphthalein alkalinities, the proportion of each may be ascertained as follows.

*Normal carbonate.* Normal carbonate is present if the alkalinity to phenolphthalein is greater than zero but less than the alkalinity to methyl orange. If the alkalinity to phenolphthalein is exactly equal to one-half the alkalinity to methyl orange, the alkalinity is due entirely to normal carbonate. If the phenolphthalein alkalinity is less than one-half the methyl orange alkalinity, normal carbonate expressed in terms of calcium carbonate is equal to twice the phenolphthalein alkalinity. If the phenolphthalein alkalinity is greater than one-half of the methyl orange alkalinity, the normal carbonate is equal to twice the difference between the methyl orange alkalinity and the phenolphthalein alkalinity.

*Hydroxide.* If hydroxide or caustic alkalinity is present, the alkalinity to phenolphthalein is greater than one-half the alkalinity to methyl orange. The alkalinity is due entirely to hydroxide, if the phenolphthalein alkalinity is equal to the methyl orange alkalinity. If the phenolphthalein alkalinity is more than half and less than all the methyl orange alkalinity, hydroxide, expressed in terms of calcium carbonate, is equal to twice the phenol-

phthalein alkalinity minus the methyl orange alkalinity.

**Bicarbonate.** Bicarbonate is present if the alkalinity to phenolphthalein is less than one-half the alkalinity to methyl orange. The alkalinity to methyl orange is due entirely to bicarbonate, if there is no phenolphthalein alkalinity. If there is phenolphthalein alkalinity, the bicarbonate in terms of calcium carbonate is equal to the methyl orange alkalinity minus twice the phenolphthalein alkalinity.

These relations of the various types of alkalinity expressed as  $\text{CaCO}_3$  are shown in Table 4.

TABLE 4.—RELATIONS BETWEEN ALKALINITY TO PHENOLPHTHALEIN AND THAT TO METHYL ORANGE IN PRESENCE OF HYDROXIDE, CARBONATE, AND BICARBONATE

Result of titration *	Value of radicle expressed in terms of calcium carbonate		
	Hydroxide	Carbonate	Bicarbonate
$P=0$	0	0	T
$P < \frac{1}{2}T$	0	$2P$	$T-2P$
$P = \frac{1}{2}T$	0	$2P$	0
$P > \frac{1}{2}T$	$2P-T$	$2(T-P)$	0
$P=T$	T	0	0

\* T = Total alkalinity in presence of methyl orange or indicator of similar range.

P = Alkalinity in presence of phenolphthalein or indicator of similar range.

#### C. CLASSIFICATION OF RESULTS

**Incrustants.** It is understood that silica, iron and aluminum oxides, and the calcium and magnesium combinations will be classed as incrusting solids.

**Non-incrustants.** The sodium compounds and other alkali combinations, as well as organic substances, will be classed as non-incrusting solids.

**Corrosive.** All acids, iron and alu-

minum sulfates, calcium chloride and nitrate, magnesium sulfate (in appreciable amount), chloride and nitrate will be classed as corrosive salts. Free carbon dioxide in appreciable amounts, and dissolved oxygen where the pH is below 10, will also fall in this class.

#### BIBLIOGRAPHY

- KAHLENBURG, L., AND LINCOLN, A. T. Solutions of silicates of the alkalies. *J. Phys. Chem.*, 2, 77 (1898).
- STABLER, H. Some stream waters of the western United States. *U. S. G. S. Water Supply Paper 274*. Govt. Printing Office, p. 165 (1911).
- PALMER, C. The geochemical interpretation of water analyses. *U. S. G. S. Bulletin No. 479*. Govt. Printing Office (1911).
- DOLE, R. B. Hypothetical combinations in water analyses. *Ind. Eng. Chem.*, 6, 710 (1914).
- COLLINS, W. D. Notes on practical water analyses. *U. S. G. S. Water Supply Paper 596-H*. Govt. Printing Office (1923).
- COLLINS, W. D. Graphic representation of analyses. *Ind. Eng. Chem.*, 15, 394 (1923).
- REES, O. W. Occurrence of silicates in natural waters. *Ind. Eng. Chem., Anal. Ed.*, 1, 200 (1929).
- AMER. RAILWAY ENGINEERING ASSN. *Manual of Recommended Practice (Water Service Section)* (1929).
- SPELLER, F. N. *Corrosion, Causes and Prevention; an Engineering Problem*. McGraw-Hill, New York (1935).
- AMER. SOC. TEST. MTLs., Method of reporting results of analysis of industrial waters. Meth. D 596-41. *A. S. T. M. Standards, III*, 814 (1942).

#### 4. Determinations

It will be observed that the methods of analysis in this text are arranged independently of the type of water being analyzed. Those interested in special types of water such as boiler feed water, boiler water, or railroad water supplies will easily find suitable methods under the appropriate con-



stituent required. The following tabulation illustrates this point.

*Boiler Water and Boiler Feed Water*

- Alkalinity
- Hydroxide—
  - Barium Chloride Method
  - Strontium Chloride Method
- Hardness—
  - Soap Method
  - Palmitate Method
- Silica—
  - Colorimetric Method
- Chloride—
  - Volhard Method
  - Mohr Method
- Sulfate—
  - Benzidene Method
  - Tetrahydroxyquinone Method
- Phosphate—
  - Colorimetric Method
- Sulfite—
  - Volumetric Method
- Oxygen, Dissolved—
  - Volumetric Method
- Oil—
  - Extraction
- pH—
  - Electrometric
- Total Dissolved Solids—
  - Calculation
  - Electrolytic Conductivity Method

*Railroad Supply Water*

- Alkalinity
- Hardness—
  - Soap Method
- Acidity
- Chloride—
  - Mohr Method
- Total Dissolved Solids—
  - Calculation
- Sulfate
- Carbon Dioxide
- pH

BIBLIOGRAPHY

AMER. RAILWAY ENGINEERING ASSN. *Manual of Recommended Practices (Water Service Section)* (1929).

5. Temperature

The temperature of the sample at the time of collection shall be expressed preferably in degrees centigrade to the nearest degree or closer if more precise data are required. The thermophone is recommended for obtaining the temperature of water at various depths below the surface.

BIBLIOGRAPHY

WARREN, H. E., AND WHIPPLE, G. C. The thermophone: a new instrument for determining temperatures. *Technology Quarterly (M. I. T.)*, 8, 125 (1895).

6. Turbidity

The turbidity of water is due to suspended matter, such as clay, silt, finely divided organic matter, microscopic organisms, and similar material. The terms "Turbidity" and "Suspended Matter" do not represent chemical equivalents. "Suspended Matter" is that portion of the residue on evaporation not in solution in the sample. "Turbidity" is an expression of an optical approximation of the suspended matter, based on the similarity of interference to the passage of light rays through a water sample when compared with standard samples of recorded turbidity.

The standard unit of turbidity is considered as that produced by one part per million of silica (diatomaceous earth or fullers' earth) in distilled water.

A. TURBIDITY MEASUREMENTS—  
STANDARD

Turbidity measurements are based on the depth of suspension required for the image of the flame of a stand-



ard candle to disappear when observed through the suspension. The standard instrument for making such measurements shall be the Jackson candle turbidimeter, which consists of a graduated glass tube, a standard candle, and a support for the candle and tube. The glass tube and the candle shall be supported in a vertical position so that the center line of the tube passes through the center line of the candle, the top of the support for the candle being 7.6 cm. (3 inches) below the bottom of the tube. The glass tube shall be graduated, preferably to read directly in turbidities, and shall have a flat polished bottom. Most of the tube should be enclosed in a metal or other suitable case when observations are being made. The candle support shall have a spring or other device so as to keep the top of the candle pressed against the top of the support. The candle shall be made of beeswax and spermaceti gauged to burn within the limits of 114 to 126 grains per hour.

To insure uniform results, it is desirable that the flame be kept as near constant size and constant distance below the glass tube as is possible. This will require frequent trimming of the charred portion of the candle string and frequent observations to see that the candle is pushed to the top of its support. Each time before lighting the candle remove such portion of the charred part of the string as is very easily broken off with the fingers. Do not keep the candle lighted for more than a few minutes at a time, for the flame has a tendency to increase in size.

The observation is made by pouring

the suspension into the glass tube until the image of the candle flame just disappears from view. Pour very slowly when the candle becomes only faintly visible. After the image has disappeared the removal of one per cent of the suspension from the tube should make it again visible. Care should be taken to keep the glass tube clean on both the inside and the outside. The accumulation of soot or moisture on the lower side of the glass bottom of the tube may interfere with the accuracy of the results.

The figures in Table 5 give the tur-

TABLE 5.—GRADUATION OF CANDLE  
TURBIDIMETER

Depth of liquid (cm.)	Turbidity	Depth of liquid (cm.)	Turbidity
2.3	1000	11.4	190
2.6	900	12.0	180
2.9	800	12.7	170
3.2	700	13.5	160
3.5	650	14.4	150
3.8	600	15.4	140
4.1	550	16.6	130
4.5	500	18.0	120
4.9	450	19.6	110
5.5	400	21.5	100
5.6	390	22.6	95
5.8	380	23.8	90
5.9	370	25.1	85
6.1	360	26.5	80
6.3	350	28.1	75
6.4	340	29.8	70
6.6	330	31.8	65
6.8	320	34.1	60
7.0	310	36.7	55
7.3	300	39.8	50
7.5	290	43.5	45
7.8	280	48.1	40
8.1	270	54.0	35
8.4	260	61.8	30
8.7	250	72.9	25
9.1	240		
9.5	230		
9.9	220		
10.3	210		
10.8	200		

bidity for the depth at which the image of the candle flame just disappears.

Alternate procedures involving the use of commercial turbidimeters may be followed provided that the equipment has been calibrated against the candle turbidimeter.

## B. TURBIDITY MEASUREMENTS—

### ABOVE 100

The standard method of measurement of turbidities from 100 to 1000 parts per million shall be the use of the candle turbidimeter as described in the preceding section. Alternate procedures involving the use of standard suspensions in bottles may be followed if the frequency of tests requires the use of such equipment, and further, provided that the readings obtained by the use of such suspensions are checked at least once daily with the candle turbidimeter.

Samples having a turbidity above 1000 parts per million shall be diluted with one or more equal amounts of turbidity-free water, until the resultant turbidity falls below 1000 parts per million. The turbidity of the original sample should be computed from the reading made on the dilute sample. For example, if the reading on the dilute sample is 500 and the amount of the original sample in the dilute sample is 1 part in 6, the turbidity of the original sample is 3000.

## C. TURBIDITY MEASUREMENTS—BETWEEN 5 AND 100

Samples having a turbidity of from 5 to 100 parts per million may be esti-

mated by comparison with standard suspensions in bottles.

Samples having a turbidity between 25 and 100 may be estimated by the use of the candle turbidimeter. In both cases commercial turbidimeters may be used providing the instruments are calibrated against the candle turbidimeter.

### 1. Preparation of Standards

1.1 Add approximately 5 g. of fullers' earth to 1 liter of distilled water, thoroughly agitate intermittently for an hour and then allow to stand 24 hours. Withdraw the supernatant without disturbing the sediment in the bottom and test the turbidity with the candle turbidimeter. Take successive portions of the suspension and dilute with distilled water until the mixtures resulting correspond to the depth of liquid reading of the various turbidity standards desired in the 5-100 range, using the candle turbidimeter for making the readings.

1.2. If fullers' earth does not produce a suspension, the color of which is reasonably similar to the suspended matter in the water under examination, suspended matter or bottom sediments from a stream may be acid treated to remove soluble constituents, washed by decantation and adjusted as above directed.

1.3 The standards, when prepared, shall be placed in clean resistant glass bottles of 1 liter capacity or greater. Enough free space at the top of the bottle shall be left to allow adequate agitation when readings are being made.

The standards shall be made fresh

at least every month and shall be kept tightly stoppered.

In order to prevent bacterial or algae growths from developing, a small amount of mercuric chloride may be added to the standards.

## 2. Procedure

The sample shall be placed in a bottle of the same type as the standards, and the standards and sample shall be well shaken. The comparison shall be made by viewing the sample and the standards sidewise, looking through both at some object and noting the distinctness with which it can be seen. The turbidity of the sample shall be recorded as that of the standard bottle which produced the visual effect most closely approximating that of the sample.

Readings will be facilitated if a series of black ruled lines on white paper be the objects viewed, and further, if the light, preferably electric, illuminates both sample and standards from above while the comparison is being made, with no direct rays from the light reaching the eye.

In using commercial turbidimeters, the manufacturer's instructions should be followed.

## D. TURBIDITY MEASUREMENTS—LESS THAN 5

When the turbidity of a sample is less than 5, estimations are best made with an instrument of the Baylis or the St. Louis type, or other available turbidimeters.

### 1. Preparation of Standards

1.1 Dilute a suspension (the turbidity of which has been observed by

the use of the candle turbidimeter to be any recorded amount above 25 and not over 100) with zero turbidity distilled water until the computed turbidity is 10 parts per million. This stock suspension should be made fresh at least every month. Bacterial and mold growths may be prevented by adding a small amount of mercuric chloride.

1.2 Dilute standards of 0.0, 0.2, 0.4, 0.6 0.8, 1.0, etc. should be made weekly. With a stock suspension having a turbidity of 10, one ml. made up to 100 ml. with distilled water will have a turbidity of 0.1 part per million. The dilute standards should be made up in amount large enough to fill the comparison tubes, but are preferably stored in resistant glass bottles when not in use.

## 2. Procedure

The comparison tubes of the particular type adapted to the interferometer being used shall be made of clear colorless glass. They shall be kept scrupulously clean, both inside and out, and should be discarded when scratched or etched. Do not handle the tubes below the point where the light strikes them.

Fill a series of tubes to the mark with the dilute standards and the samples—all thoroughly agitated. Allow air bubbles to escape. View from above and match the sample with the standard giving the same or approximately the same interference effect. Record in terms of the turbidity of the matched standard.

In using commercial turbidimeters, the manufacturer's instructions should be followed.



## E. RECORDING TURBIDITY READINGS

The results of turbidity observations shall be expressed in whole numbers which correspond to units of turbidity as follows:

Turbidity Between		Record to Nearest
0 and	1	tenth
1 "	10	unit
11 "	100	5
101 "	400	10
401 "	700	50
701 "	greater	100

## BIBLIOGRAPHY

- WHIPPLE, G. C., AND JACKSON, D. D. A comparative study of the methods used for the measurement of turbidity of water. *Technology Quarterly (M. I. T.)*, 13, 274 (1900).
- Report of committee on standard methods of water analysis. *Pub. Health Papers and Repts. Am. Pub. Health Assn.*, 27, 377 (1901).
- WELLS, P. V. Turbidimetry of water. *J. Amer. W. W. Assn.*, 9, 488 (1922).
- BAYLIS, J. R. Turbidimeter for accurate measurement of low turbidities. *Ind. Eng. Chem.*, 18, 311 (1926).
- WELLS, P. V. The present status of turbidity measurements. *Chem. Rev.*, 3, 331 (1927).
- BAYLIS, J. R. Turbidity determinations. *W. Wks. and Sew.*, 80, 125 (1933).

## 7. Color

The "color" or the "true color" of water shall be considered the color that is due only to substances in solution; that is, it is the color of the water after the suspended matter has been removed. The accurate determination of color in water containing matter in suspension is impossible. The removal of suspended matter by centrifuging before the color obser-

vation is made gives the best results. No filter shall be used since filters exert marked decolorizing action.

The "apparent color" shall be considered as including not only the true color, but also any color produced by substances in suspension. The apparent color, if determined, shall be determined on the original sample without filtration.

The platinum-cobalt method of measuring color shall be considered as the standard, and the unit of color shall be that produced by 1 mg. of platinum per liter. The ratio of cobalt to platinum may be varied to match the hue in special cases. The proportion given below is usually satisfactory. The true and apparent color of clear water or waters with low turbidities is substantially the same.

The results of color determination shall be expressed in whole numbers and recorded as follows:

Color Between		Record to Nearest
1 and	50	unit
51 "	100	5
101 "	250	10
251 "	500	20

## A. COMPARISON WITH PLATINUM-COBALT STANDARDS

### 1. Preparation of Standards

1.1. Dissolve 1.245 g. of potassium chloroplatinate ( $K_2PtCl_6$ ) containing 0.5 g. of platinum, and 1 g. of crystallized cobaltous chloride ( $CoCl_2 \cdot 6H_2O$ ), containing approximately



0.248 g. of cobalt, in water with 100 ml. of concd. HCl, and dilute to 1 liter with distilled water. This solution has a color of 500.

1.2. In the absence of a reliable supply of potassium chloroplatinate, chloroplatinic acid may be substituted, as follows: dissolve 0.5 g. of metallic platinum in aqua regia; remove nitric acid by repeated evaporation to dryness after adding an excess of hydrochloric acid. Dissolve this product with 1 g. of cobalt chloride as directed in 1.1.

1.3. To prepare standards having colors of 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, and 70, dilute 0.5, 1, 1.5 ml. etc. of the above solution with distilled water to 50 ml. in standard Nessler tubes. If 100 ml. standard Nessler tubes are used, colors of 5, 10, etc. are obtained by diluting 1, 2, etc. ml. of the standard solution to 100 ml. with distilled water. Protect the tubes from evaporation and from dust when not in use (Part I, Sec. 49, A, 3.8, page 97).

## 2. Procedure

Waters having a color greater than 70 shall be diluted with distilled water before making the comparison, in order that no difficulty may be encountered in matching hues.

The color of a sample shall be observed by filling a standard Nessler tube to a height equal to that in the standard tubes with the water to be examined and by comparing it with standards. The observation shall be made by looking vertically downward through the tubes on a white or mirrored surface placed at such an angle

that light is reflected upward through the column of liquid.

Inasmuch as the proportions of the standard color solution in the comparison tubes are such as to represent an aliquot part of a liter, the readings are direct as parts per million.

## B. COMPARISON WITH GLASS DISCS

Since the platinum-cobalt standard method is not well adapted for field work, the color of water to be tested may be compared with that of glass discs held at the end of metallic tubes through which they are viewed by looking toward a white surface. The glass discs are individually calibrated to correspond with colors on the platinum scale. Experience has shown that the glass discs used by the United States Geological Survey give results in substantial agreement with those obtained by the platinum determinations, and their use is recognized as a standard procedure.

The use of prepared glass or liquid standards in the laboratory is permissible only when they have been properly checked against the standards listed in A above.

## BIBLIOGRAPHY

- HAZEN, A. A new color standard for natural waters. *Am. Chem. J.*, 14, 300 (1892).  
FITZGERALD, D., AND FOSS, W. E. The color of water. *J. Franklin Inst.*, 138, 401 (1894).  
HAZEN, A. The measurement of the colors of natural waters. *J. Am. Chem. Soc.*, 18, 264 (1896).  
U. S. Geological Survey. Measurement of color and turbidity in water. *U. S. Geol. Survey, Division of Hydrography, Circ. 8* (1902).

## 8. Odor

Odors in water may be associated with the presence of pollution or otherwise objectionable substances, e.g., decomposing organic material, plankton, or industrial wastes. Determination of odor quality should be the first step in any endeavor to prevent this cause of consumer complaint.

Odors in water are caused by volatile substances in concentrations generally too small to be detected by ordinary analytical methods, hence reliance is placed on the sense of smell. With all its deficiencies, the olfactory sense is a most sensitive device for detecting small concentrations of odor, but unfortunately, lacks precision.

There is no absolute odor value. Different persons react differently to the same concentration of odor and one individual may report a different intensity for the same odor at different times. Therefore, it follows that an odor value obtained in one comparison should not be removed and used independently in some later comparison. If necessary, any daily or weekly fluctuation in the observer's sensitivity can be checked by making a periodic threshold test on a freshly prepared solution of a standard odorous substance, e.g., phenol. This refinement is seldom needed in routine plant operation.

### 1. Reagents and Apparatus

1.1. Odor-free water: prepared by passing tap water through a train of two 4 liter bottles, containing granular activated carbon. The bottles are interconnected by glass tubing, the inlet extending above the carbon and the outlet extending from the bottom

of the bottle. The flow of water should be less than 5 gallons per hour.

1.2. One dozen 500 ml. glass-stoppered Erlenmeyer flasks, each flask given a serial number. Glassware must be thoroughly cleaned and then rinsed several times with odor-free water before each use.

1.3. Chemical thermometer (0-110° C.).

1.4. Ten ml. Mohr pipette; 25 ml. graduated cylinder; 50 ml. graduated cylinder; 100 ml. graduated cylinder; 200 or 250 ml. graduated cylinder.

1.5. One liter glass-stoppered bottles to hold samples of water being examined.

1.6. Nosepiece (optional): A glass osmoscope may be prepared from  $\frac{3}{4}$  in. tube 8 in. to 12 in. long, open at lower end, and terminating at upper end in an enlarged section that will cover outside of nostrils. If an osmoscope is used it must be thoroughly rinsed between each and every observation.

## 2. Precautions

Certain conditions are required to obtain consistent results.

Some practice with the test is desirable to develop consistent sensitivity of the sense of smell.

The odor-free water when prepared should be truly free of all detectable odor.

All glassware must be free of odor. This is accomplished by thorough cleansing followed by several rinses with odor-free water.

All dilutions when examined for odor should be at a uniform temperature, deviation not to exceed 1° C.

Each dilution should be compared with an odorless standard. This sim-

plifies the work of the observer since his task then is to decide only whether or not an odor is present in the dilution. The odor-free standard checks judgment and minimizes any reliance on odor memory.

A sudden change in the character of the odor during the testing procedure should be considered as a warning that there may be interference from outside odors or that the diluting water may not be odor-free. The character of odor should always be recorded for future consideration.

To eliminate psychological influences, the samples are coded, before testing, and dilutions are examined in a manner to prevent the observer knowing what odor concentration is being observed.

The test should be conducted in a room from which outside odors are absent.

The test should not be prolonged to a point where the sense of smell becomes fatigued.\* The total time of the test should not exceed one hour.

### 3. Procedure

Obtain the approximate range of odor value. To one Erlenmeyer flask add 50 ml. of water sample, to a second flask add 14 ml., to a third flask add 5 ml. Add sufficient odor-free water to each flask to make a total volume of 200 ml., and also add 200 ml. of odor-free water to a fourth flask which will be the reference for comparison.

Determination can be made of either the cold odor or the hot odor.

*Cold Odor:* Bring dilutions to tem-

perature of 24°–25° C.—the exact temperature is not important but it is essential that a uniform temperature be used throughout.

*Hot Odor:* Temperatures of 50°, 60°, and 65° C. have been successfully used by different operators. The best temperature to use is a matter of individual preference. Heat samples to desired temperature on hot plate or water bath. As with cold odor, a uniform temperature must be used and should not deviate over 1°.

Shake each flask three or four times uniformly before smelling for odor. Observer should characterize type of odor. Precision of observation is increased if odor-free water is smelled alternately with each dilution.

Note which flasks contain an odor and which do not, and according to the results obtained, prepare intermediate dilutions, in each case using sufficient odor-free water to make a total volume of 200 ml.

Arrange flasks so their identity is unknown and bring to desired temperature. Then take up flasks one by one and observe for odor. Place those flasks in which an odor is found into one group, and those without an odor into another group. Record results as "plus" or "zero" for each dilution.

The results are reported in threshold odor numbers. The threshold odor number is calculated from the amount of sample in the most diluted portion which gives perceptible odor. The volume of the dilution (200 ml.) divided by the volume of the sample in the dilution equals the threshold odor number. For example: 5 ml. diluted to 200 ml. is the most dilute portion giving perceptible odor.

\* In water characterized by a strong odor, it is desirable to begin with dilutions below the detectable threshold.

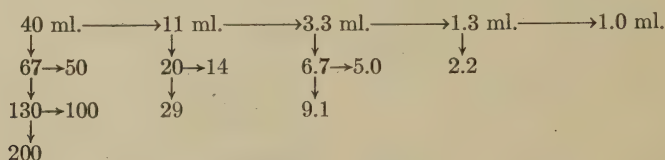


$200 \div 5 = 40$ , is the threshold odor number.

The extent to which intermediate dilutions are made will depend on the ability of the operator to detect small increments in odor, and on the purpose for which the odor determinations are made.

The threshold odor number should not be confused with the threshold odor concentration. The threshold odor concentration is the smallest amount of odor producing material in ppm. required to give a perceptible odor. If the threshold odor concentration is known, that value multiplied by the threshold odor number will give the concentration of odor producing material in the sample.

Experience will enable an operator to examine simultaneously two water samples—in which case a duplicate set of flasks is required. For simul-



THRESHOLD ODOR CHART

taneous examination of more than two samples use Procedure 4.

#### 4. *Alternate Procedure for Examining Several Water Samples*

When two operators are available, the simultaneous examination of two or more samples can be conducted in a shorter period of time by a parallel procedure. The samples are labeled (e.g., A, B, C, D, E, but not over five samples should be run at one time).

One operator (the diluter) prepares a dilution by adding 40 ml. of sample

A plus 160 ml. odor-free water (200 ml. total) to one flask, and 200 ml. odor-free water to another flask. Both flasks are brought to the desired temperature (see Procedure 3 above), and given—without identification—to the other operator (the observer). The diluter then prepares a similar dilution of samples, B, C, etc.

After noting observer's reaction with first dilution of sample A, the diluter refers to "Threshold Odor Chart" for further dilutions. In every case sufficient odor-free water is added to make a total volume of 200 ml.

#### *Threshold Odor Chart*

Start with 40 ml. When odor is positive follow arrow to right for ml. for next dilution. When no odor is found, follow lower arrow and prepare dilution below.

The second dilution of sample B is prepared on the same basis—and procedure continues with further dilutions until the threshold odor number of each sample is found.

Interpretation of results by parallel method is illustrated in the following recorded results.

	Order of preparation of dilution	Ml. of sample used	Odor	Threshold odor Number
Sample A	1	40	0	
	3	67	+	
	5	50	+	→ 4.



Sample B	2	40	+	→10.
	4	11	0	
	6	20	+	
	7	14	0	

It is desirable to have at least 2 positive and 2 negative values. Therefore, the threshold odor number of sample A should be confirmed by repeating dilution No. 1.

### 5. Type of Odor

It is desirable to observe the characteristic of hot or cold odor by a descriptive term assignable from the list in Table 6. For record purposes the code letters may be used.

### BIBLIOGRAPHY

WHIPPLE, G. C. The observation of odor as an essential part of water analysis. *Pub. Health Papers and Repts. Am. Pub. Health Assn.*, 25, 587 (1899).

ZWAARDEMAKER, H. Odoriferous materials. *International Critical Tables*, I, 358 (1926).

SPAULDING, C. H. The quantitative determination of odor in water. *Am. J. Pub. Health*, 21, 1038-39 (1931).

BAYLIS, J. R. Procedure for making odor and taste determinations. *W. Wks. & Sew.*, 79, 425 (1932).

FAIR, G. M. On the determination of odors and tastes in water. *J. New Eng. W. W. Assn.*, 47, 248 (1933).

FAIR, G. M., AND WELLS, W. F. The air dilution method of odor determination in water analysis. *J. Amer. W. W. Assn.*, 26, 1670 (1934).

FAIR, G. M., AND MOORE, E. W. Measurement of intensity and concentration of odors associated with sewage treatment processes. *Sew. Wks. J.*, 7, 182 (1935).

CROCKER, E. C. Seeking a working language for odors and flavors. *Ind. Eng. Chem.*, 27, 10, 1225 (1935).

HULBERT, R., AND FEBEN, D. Studies on accuracy of threshold odor value. *J. Amer. W. W. Assn.*, 33, 1945 (1941).

SPAULDING, C. H. Accuracy and application of threshold odor test. *J. Amer. W. W. Assn.*, 34, 877 (1942).

*Taste and Odor Control in Water Purification*. Published by Industrial Chemical Sales Div., W. V. Pulp & Paper Co. (1942).

TABLE 6.—ODOR CHARACTERISTICS

Code	Nature of odor	Description
A	Aromatic (Spicy)	Such as odors of camphor, cloves, lavender and lemon.
Ac	Cucumber	Such as odor of <i>Synura</i> .
B	Balsamic (Flowery)	Such as odors of geranium, violets and vanilla.
Bg	Geranium	Such as odor of <i>Asterionella</i> .
Bn	Nasturtium	Such as odor of <i>Aphanizomenon</i> .
Bs	Sweetish	Such as odor of <i>Coelosphaerium</i> .
Bv	Violets	Such as odor of <i>Mallomonas</i> .
C	Chemical	Such as odors due to industrial wastes or chemical treatment.
Cc	Chlorinous	Odor of free chlorine.
Ch	Hydrocarbon	Such as odors of oil refinery wastes.
Cm	Medicinal	Such as odors of phenol or iodoform.
Cs	Sulfuretted	Odor of hydrogen sulfide.
D	Disagreeable	Pronounced unpleasant odors.
Df	Fishy	Such as odors of <i>Uroglenopsis</i> and <i>Dinobryon</i> .
Dp	Pigpen	Such as odor of <i>Anabaena</i> .
Ds	Septic	Such as odor of stale sewage.
E	Earthy	Such as odor of damp earth.
Ep	Peaty	Such as odor of peat.
G	Grassy	Such as odor of crushed grass.
M	Musty	Such as odor of decomposing straw.
Mm	Moldy	Such as odor of a damp cellar.
V	Vegetable	Such as odor of root vegetables.

THOMAS, H. A., JR. Calculation of threshold odor. *J. Amer. W. W. Assn.*, 35, 751 (1943).

## 9. Residue

### A. RESIDUE ON EVAPORATION

Total residue on evaporation or total solids includes both suspended and dissolved solids. The total solids weighed will vary with the temperature of drying. A fixed temperature must be adhered to for reproducible results; 103° C. has been selected. Special problems may require the use of other drying temperatures.

Total fixed residue represents the residue remaining after ignition of the total residue. Loss on ignition represents not only organic matter but volatile decomposition products from carbonates, nitrates and other components of the residue. Loss on ignition is a relative, not a precise determination.

#### 1. Procedure for Total Residue and Total Fixed Residue

1.1. Total residue. Evaporate a suitable size sample, thoroughly shaken and unfiltered, in a weighed dish, preferably platinum, on a water or steam bath. In determining the residue on evaporation from a sample of condensate, extreme care must be used to secure accurate results. Special apparatus is recommended in this case. The original literature should be considered for details. Dry the residue at 103° C. for one hour. In some cases, one hour will not be sufficient drying time to secure constant weight. In such cases, drying shall be continued to constant weight. Report the increase in weight as total solids or residue on evaporation.

1.2. Total fixed residue. Ignite the residue remaining in the dish at a low red heat, noting any significant color change or odor. If greater accuracy is desired, this should be completed in an electric muffle furnace. Allow the dish to cool, moisten the residue with distilled water, dry at 103° C., cool and weigh. The amount thus recorded represents fixed residue. The difference between this figure and that recorded as total residue represents loss on ignition.

#### 2. Procedure for Dissolved Residue

2.1. Total dissolved residue. For this determination the sample should be filtered, if necessary, so that the suspended matter remaining is less than 1 ppm. in terms of turbidity. Evaporate a suitable size sample in a weighed dish, preferably platinum, on a water or steam bath. Dry the residue at 103° C. for one hour. In some cases, one hour will not be sufficient drying time to secure constant weight. In such cases, drying shall be continued to constant weight. Report the increase in weight as total dissolved solids or residue.

2.2. Fixed dissolved residue. Ignite the residue remaining in the dish at a low red heat noting any significant color change or odor. If greater accuracy is desired, this should be completed in an electric muffle furnace. Allow the dish to cool, moisten the residue with distilled water, dry at 103° C., cool and weigh. The amount thus recorded represents dissolved fixed residue. The difference between this figure and that recorded as total dissolved residue represents loss on ignition.

### 3. Procedure for Suspended Residue

3.1. Total suspended residue. Prepare a mat of acid and alkali washed asbestos fiber 2 mm. thick in a Gooch crucible. Dry in an oven for one hour at 103° C., cool and weigh. Filter a 1 liter sample having a turbidity of 50 ppm. or less. If the turbidity is higher, use sufficient water to obtain 50 to 100 mg. of suspended residue. Dry for one hour at 103° C., cool in a desiccator and weigh promptly when cool. In some cases, one hour will not be sufficient drying time to secure constant weight. In such cases, drying shall be continued to constant weight. Report the increase in weight as total suspended residue or total suspended solids.

The difference between the total residue or solids in filtered and unfiltered portions of a sample may be used as a basis for calculating the suspended residue.

3.2. Fixed suspended residue. Ignite the residue remaining in the crucible at a low red heat, noting any significant color change or odor. If greater accuracy is desired, ignition should be completed in an electric muffle furnace. Allow the dish to cool, moisten the residue with distilled water, dry at 103° C., cool and weigh. The amount thus recorded represents suspended fixed residue. The difference between this figure and that recorded as total suspended residue represents loss on ignition.

### B. RESIDUE BY ELECTROLYTIC CONDUCTIVITY—TOTAL DISSOLVED SOLIDS

In dilute solution, particularly where the composition of the sample does not vary greatly, the electrolytic

conductivity can be used to measure the dissolved solids present. Over a limited range the relationship is linear. Since gases, such as ammonia and carbon dioxide, dissolve to form electrolytes, they must be removed or corrected for in cases where their presence will introduce appreciable error, e.g., in condensate.

#### 1. Apparatus

1.1. Electrolytic conductivity alternating current bridge.

1.2. Dip-type conductivity cells, platinum electrodes.

1.3. Standard solution for conductivity measurement. Potassium chloride standard solution, 0.01 N. Dissolve 0.7453 g. of KCl (weighed in vacuo) per 1000 g. of solution. At 25° C. or 77° F. the specific conductivity of this solution is 1408.8 micromhos. Subtract the specific conductivity of the water used for making the solution from the value given.

#### 2. Determination of Cell Constant of Conductivity Cell

Measure the conductivity of the standard solution of potassium chloride at constant temperature. Compensate for temperature differences from 25° C. by a temperature compensator on the conductivity bridge or calculate the value to 25° C. Correct the cell constant to obtain accordance with the established value for the solution.

#### 3. Calibration

Determine the total dissolved solids in the sample gravimetrically. Add small increments of the solution to the purest water available, preferably



degassed condensed steam, and determine the electrolytic conductivity. Plot or tabulate ppm. of solids vs. electrolytic conductivity.

#### 4. *Special Procedure for Condensed Steam*

4.1. For accurate results carbon dioxide and ammonia should be removed prior to determination of the electrolytic conductivity. Since complete removal may not be secured, both constituents should be determined in the condensate.

4.2. Use a sampling nozzle corresponding to the design recommended by the Power Test Code Committee of the American Society of Mechanical Engineers. Use small diameter tubing of  $\frac{1}{16}$  in. or  $\frac{1}{8}$  in. I. D. of the proper length to obtain the desired flow. Use stainless steel for sampling nozzle, valve and tubing. Copper tubing may be used at pressures less than 250 psi. gage. Insulate the tubing to prevent condensation. The cooling coil may be made of block tin or copper tubing.

4.3. Two methods for degasification of the condensed steam are stripping at atmospheric pressure and elevated temperatures and stripping at sub-atmospheric pressures and elevated temperatures. The operation of such equipment should be obtained from the original articles.

#### 5. *Calculation of Total Dissolved Solids*

Determine the ammonia and carbon dioxide in the condensed steam by the procedures described in Part I, Sec. 32 and Sec. 14, pages 64 and 32. Since carbon dioxide is usually left

in negligible amounts, a correction for ammonia only need be applied. A frequently used value is 0.9 micromho which is subtracted for each part per million of ammonia nitrogen present. Then also subtract the value of 0.05 micromho, that of pure water, from the measured specific conductivity. Compute the total dissolved solids by applying the calibration factor previously obtained. A value of 0.5–0.6 ppm. solids per micromho is one widely obtained. If ammonia and carbon dioxide are both present in the sample, the procedure described by Watson should be employed to obtain a single conductivity correction.

#### C. RESIDUE BY CALCULATION—TOTAL DISSOLVED SOLIDS

For routine control the total dissolved solids may be estimated by adding the concentration of all the compounds presumably present as determined from an analysis of the sample. The value so obtained should be multiplied by a numerical factor obtained by comparison of calculated and gravimetrically determined values. By the use of such a calibration factor, secured on a number of samples spread over a reasonable period of time, the total dissolved solids may be calculated with reasonable accuracy. An illustration of such a calculation follows:

Total Dissolved Solids = Factor  $\times$  (total alkalinity as ppm.  $\text{CaCO}_3$  + ppm.  $\text{Na}_2\text{SO}_4$  + ppm.  $\text{NaCl}$ ).

#### BIBLIOGRAPHY

ZINZALIAN, G., AND WITTHROW, S. R. Total solids in natural brines. *Ind. Eng. Chem. Anal. Ed.*, 4, 210 (1932).



- HOWARD, C. S. Determination of total dissolved solids in water. *Ind. Eng. Chem. Anal. Ed.*, 5, 4 (1933).
- JONES, G., AND BRADSHAW, B. C. The measurement of the conductance of electrolytes. V. A redetermination of the conductance of standard potassium chloride solutions in absolute units. *J. Am. Chem. Soc.*, 55, 1780-1800 (1933).
- POWELL, S. T. Steam contamination. *Combustion*, 9, 5, 25-31 (1937).
- POWELL, S. T., BACON, H. E., JR., MCCHESENEY, I. G., AND HENRY, F. Design and development of apparatus for measurement of steam quality by electrical conductivity methods. *Trans. Am. Inst. Chem. Engrs.*, 33, 116-38 (1937).
- ULMER, R. C. Determination by the evaporation method of small amounts of dissolved solids in water, such as condensed steam from boilers. *Proc. A. S. T. M.*, 39, 1221-32 (1939).
- SCHWARTZ, M. C., GURNEY, W. B., AND CROSSAN, T. E. Determination of the purity of steam by gravimetric and spectrographic methods. *Trans. A. S. M. E.*, 62, 718-22 (1940).
- GURNEY, W. B., SCHWARTZ, M. C., AND CROSSAN, T. E. Determination of purity of steam by electrolytic conductivity method. *Trans. A. S. M. E.*, 62, 728-33 (1940).
- STRAUB, F. G., AND NELSON, E. E. A new degasifying steam condenser for use in conductivity determination. *Trans. A. S. M. E.*, 63, 645-53 (1941).
- WATSON, A. Discussion. *Trans. A. S. M. E.*, 62, 728-33 (1940), PARTRIDGE, E. P. Discussion, and MCKINNEY, D. S. Calculation of corrections to conductivity measurements for dissolved gases. *Proc. A. S. T. M.*, 41, 1296 (1941).

## 10. Hardness

Water which requires an excessive amount of soap to form a lather or forms much incrustation on vessels in which it stands or is heated is commonly called "hard water." Calcium and magnesium, and iron and aluminum to a less extent, are responsible for these effects. (The pseudo-hardness of brines is caused by their high sodium content which prevents solution of sodium soaps.)

Hardness is expressed in terms of calcium carbonate. The calcium carbonate equivalent of the calcium and magnesium content, sometimes with that of iron and aluminum, is a measure of the total hardness of a water. The soap and soda reagent methods given below are approximate tests for estimating this value; ordinary quantitative methods are more reliable.

When the total hardness is greater than the carbonate and bicarbonate alkalinity, the amount of hardness equivalent to the alkalinity is called carbonate hardness and the amount of hardness in excess of this is called non-carbonate hardness. When the total hardness is equal to or less than the sum of the carbonate and bicarbonate alkalinity, there is no non-carbonate hardness; the total hardness is all carbonate hardness. (See Part I, Sec. 3, B, 2-3, page 7.)

### A. TOTAL HARDNESS BY CALCULATION

The most accurate method for ascertaining total hardness is to compute it from the results of the determination of calcium (Part I, Sec. 28, page 59), and magnesium (Part I, Sec. 29, page 60). Iron (Part I, Sec. 24, page 51) and other metals must be included in the calculation if they are present in significant amounts.

Total Hardness as ppm.  $\text{CaCO}_3 = 2.497 \times \text{ppm. Ca} + 4.115 \times \text{ppm. Mg}$ .

### B. HARDNESS BY PALMITATE—TOTAL, MAGNESIUM AND CALCIUM

*Total Hardness.* Potassium palmitate precipitates calcium and magnesium from solution as calcium and magnesium palmitates. The addition of an excess of potassium palmitate

over that required for the complete precipitation of calcium and magnesium palmitates produces, by hydrolysis, a solution alkaline to phenolphthalein. The method is considered to be accurate to within 3 to 5 ppm. as  $\text{CaCO}_3$ .

*Magnesium Hardness.* Calcium is first removed from a solution containing both calcium and magnesium by precipitation as calcium oxalate. The addition of an excess of potassium palmitate over that required for the precipitation of magnesium palmitate produces, by hydrolysis, a solution alkaline to phenolphthalein.

*Calcium Hardness.* The calcium hardness may be secured by difference between the total hardness and the magnesium hardness both of which are determined experimentally.

### 1. Reagents

1.1. Hydrochloric or sulfuric acid, 0.02 N.

1.2. Sodium hydroxide, 0.02 N.

1.3. Methyl orange indicator solution.

1.4. Phenolphthalein indicator solution.

1.5. Potassium palmitate solution, 0.04 N.

Dissolve 10.25 g. of palmitic acid in 500 ml. 95 per cent ethyl alcohol. Warm on a steam bath to effect solution. Add 50 ml. of a solution of 40 g. KOH in 1 liter of 95 per cent ethyl alcohol. While still on the steam bath add 420 ml. of warm (approximately  $50^\circ \text{C}$ .) glycerine with constant stirring. Cool, add 2 ml. phenolphthalein solution and titrate to a very faint pink with the alcoholic KOH. Dilute

to 1 liter at room temperature with 95 per cent ethyl alcohol.

Standardize potassium palmitate solution by following the procedure for total hardness with exception that it is not necessary to remove the alkalinity.

1.6. Standard calcium chloride solution. Dissolve 0.5 g. of calcium carbonate with a small amount of dil. HCl, being careful to avoid spattering. Wash down with  $\text{CO}_2$  free distilled water and neutralize with dil.  $\text{NH}_4\text{OH}$  to a slight alkalinity, using litmus as an indicator. Make up to 500 ml. with  $\text{CO}_2$  free distilled water and store in a glass stoppered bottle. One ml. of this solution is equivalent to 1 mg. of calcium carbonate.

Mg.  $\text{CaCO}_3$  per ml. potassium palmitate = ~~0.5~~ ml. standard calcium <sup>chloride</sup> soln.]  $\div$  ml. potassium palmitate soln.

1.7. Sodium oxalate solution, saturated.

### 2. Procedure

2.1. Total hardness. Dilute a small sample with distilled water before proceeding if the hardness is known to be greater than 800 ppm. (as  $\text{CaCO}_3$ ). To a 50 ml. sample, add 0.5 ml. of 0.02 N HCl or  $\text{H}_2\text{SO}_4$  in excess of that required to neutralize the alkalinity and boil for one minute to expel the carbon dioxide. Cool and add 0.5 ml. of phenolphthalein solution. Neutralize with 0.02 N NaOH, then add 0.02 N HCl to the acid side of phenolphthalein. Add potassium palmitate solution until a faint but permanent pink color is produced.

8  
Error  
Corrected  
CJDB.

Total Hardness as ppm.  $\text{CaCO}_3 = [1000 \times \text{ml. palmitate soln.} \times \text{mg. CaCO}_3 \text{ per ml. palmitate soln.}] \div \text{ml. sample.}$

**2.2 Magnesium hardness.** To a 50 ml. sample, add 0.5 ml. of 0.02 N HCl or  $\text{H}_2\text{SO}_4$  in excess of that required to neutralize the alkalinity and boil for one minute to expel the carbon dioxide. Add 1 ml. of  $\text{Na}_2\text{C}_2\text{O}_4$  soln. and boil for one minute. Cool and add 0.5 ml. of phenolphthalein solution. Neutralize with 0.02 N NaOH, then add 0.02 N HCl to the acid side of phenolphthalein. Add potassium palmitate solution until a faint but permanent pink color is produced.

Magnesium Hardness as ppm.  $\text{CaCO}_3 = [1000 \times \text{ml. palmitate soln.} \times \text{mg. CaCO}_3 \text{ per ml. palmitate soln.}] \div \text{ml. sample.}$

**2.3. Calcium hardness.** Calculate the calcium hardness by difference.

Calcium Hardness as ppm.  $\text{CaCO}_3 = [\text{Total hardness as CaCO}_3 - \text{Mg hardness as CaCO}_3] = [1000 \times (\text{ml. palmitate soln. (total)} - \text{ml. palmitate soln. (Mg)}) \times \text{mg. CaCO}_3 \text{ per ml. palmitate soln.}] \div \text{ml. sample.}$

### C. HARDNESS BY SOAP—TOTAL, CALCIUM, MAGNESIUM, AND LOW HARDNESS (ZEOLITE EFFLUENT)

**Total Hardness.** The determination of hardness by the soap method roughly approximates the amount of calcium and magnesium in a water, though it actually measures the soap-consuming power of the water.

**Calcium Hardness.** In the presence of ammonium chloride, in a solution whose pH is adjusted between 11.7 and 12.0, soap solution will react only with calcium.

**Magnesium Hardness.** The difference between the quantity of soap

solution required to obtain the total hardness and that required for calcium is taken as the quantity of soap solution which is equivalent to the magnesium compounds present.

**Low Hardness (Zeolite Effluent).** In order to use a reasonable volume of soap solution for such waters, a dilute soap solution should be used.

#### 1. Reagents

**1.1. Standard calcium chloride solution** (Part I, Sec. 10, B, 1.6, page 24). Dissolve 0.5 g. of pure calcium carbonate with a small amount of dil. HCl, being careful to avoid spattering. Wash down with  $\text{CO}_2$ -free distilled water and neutralize it with  $\text{NH}_4\text{OH}$  to slight alkalinity using litmus as an indicator. Make up to 500 ml. with  $\text{CO}_2$ -free distilled water and store in a glass stoppered bottle. One ml. of this solution is equivalent to 1 mg. calcium carbonate.

**1.2. Standard soap solution.** Dissolve with vigorous shaking approximately 100 g. of pure powdered castile soap in 1 liter of 80 per cent ethyl alcohol. Let this solution stand, at least over night, then decant. The stock soap solution is approximately 9 to 10 times as strong as the dilute standard soap solution. Take a portion of the stock solution, dilute with 80 per cent ethyl alcohol until, when titrated against  $\text{CaCl}_2$  soln., 1 ml. of the resulting dilution is equivalent to 1 ml. of the standard  $\text{CaCl}_2$  soln., making due and recorded allowance for the lather factor of the adjusted soap solution from at least five determinations.

The term lather factor may be defined as the amount of standard soap



solution required to produce a permanent lather in a 50 ml. portion of distilled water. Use  $\text{CO}_2$ -free water for lather factor determinations and for standardization of the solution. One ml. of this solution after subtracting the lather factor is equivalent to 1 mg. calcium carbonate. If the same water is frequently analyzed it will be of assistance to standardize the soap solution against a mixture of calcium and magnesium salts, the relative proportions of which approximate those found in the water. The strength of the soap solution should be determined from time to time, to make sure that it has not changed materially.

1.3. Sulfuric acid, 0.02 N.

1.4. Sodium carbonate, 0.02 N.

1.5. Ammonium chloride solution.

Dissolve 10 g. of  $\text{NH}_4\text{Cl}$  in 100 ml. of distilled water.

1.6. Sodium hydroxide solution, 1.0 N, carbonate-free.

1.7. Phenolphthalein indicator.

1.8. Methyl orange indicator.

1.9. Soap solution, dilute. Mix equal volumes of standard soap solution and ethyl alcohol. Standardize as under 1.2 above.

## 2. Procedure

2.1. Total hardness. Adjust the alkalinity of the sample, either with standard acid or base, so that it is just pink to phenolphthalein. Place a 50 ml. sample in an 8 ounce stoppered bottle. Add the standardized soap solution in small amounts at a time, shaking vigorously after each addition, until a strong permanent lather is secured, which will stand for five minutes with the bottle laid on its side.

To avoid mistaking the false endpoint for the true one, read the burette after the titration is apparently finished and add about 0.5 ml. more of soap solution. Continue adding soap solution if the lather disappears. Usually the false lather persists for less than five minutes.

It is usually satisfactory to add amounts of soap solution equal to the lather factor as the first additions and then, as the end point is approached, to cut the additions of soap solution to 0.1 to 0.2 ml., depending on the accuracy desired and the experience of the manipulator. If more than 7 ml. of soap solution is required, use an aliquot sample.

Total Hardness as ppm.  $\text{CaCO}_3 = [(\text{ml. of soap soln.} - \text{lather factor in ml.}) \times \text{mg. CaCO}_3 \text{ per ml. soap soln.} \times 1000] \div \text{ml. sample.}$

2.2. Calcium hardness. Adjust the alkalinity of the sample, either with standard acid or base, so that it is just pink to phenolphthalein. Place a 50 ml. sample in an 8 ounce bottle. Add 1.0 ml. of ammonium chloride solution and then 3.0 ml. of 1.0 N NaOH. Add standardized soap solution in small amounts at a time, shaking vigorously after each addition, until a strong permanent lather, which will last for one minute, is secured. If more than 7 ml. of soap solution is required, use an aliquot sample.

Calcium Hardness as ppm.  $\text{CaCO}_3 = [(\text{ml. soap soln.} - \text{lather factor in ml.}) \times \text{mg. CaCO}_3 \text{ per ml. soap soln.} \times 1000] \div \text{ml. sample.}$

2.3 Magnesium hardness. Determine the total and calcium hardness



as described. Record the ml. of soap solution consumed at both the calcium and total hardness end-points as  $V_1$  and  $V_2$  respectively.

Magnesium Hardness as ppm.  $\text{CaCO}_3$   
 $= [(V_2 - V_1) \times \text{mg. CaCO}_3 \text{ per ml. soap} \times 1000] \div \text{ml. sample.}$

2.4. Low hardness (Zeolite effluent). Adjust the alkalinity of the sample, if necessary, either with standard acid or base, so that it is just pink to phenolphthalein. Place a 50 ml. sample into an 8 ounce stoppered bottle. Add the dilute soap solution in small amounts at a time, shaking vigorously after each addition, until a permanent lather, which will last for five minutes with the bottle laid on its side, is secured.

Total Hardness as ppm.  $\text{CaCO}_3 = [(\text{ml. soap soln.} - \text{lather factor in ml.}) \times \text{mg. CaCO}_3 \text{ per ml. soap soln.} \times 1000] \div \text{ml. sample.}$

#### D. HARDNESS BY SODA REAGENT

*Non-carbonate hardness.* The addition of soda reagent, a mixture of sodium hydroxide and sodium carbonate, after the removal of carbon dioxide, precipitates calcium and magnesium from the sample as the carbonate and hydroxide respectively. The filtrate, and a blank of distilled water, both originally treated with the same amount of soda reagent, are titrated with sulfuric acid. The difference represents an amount equivalent to the non-carbonate hardness of the sample. The use of soda reagent involves an error due to the solubility of the salts of calcium and magnesium.

*Total Hardness.* The addition of sulfuric acid, prior to the addition of soda reagent, permits all the calcium

and magnesium in the sample to react with that reagent. The filtrate, and a blank of distilled water, both originally treated with the same amount of soda reagent, are similarly titrated with sulfuric acid. The difference represents an amount equivalent to the total hardness of the sample.

#### 1. Reagents

1.1. Soda reagent. Dissolve 2 g. of sodium hydroxide and 2.65 g. of anhydrous sodium carbonate in distilled water, mix and make up to 1 liter. The solution is approximately 0.1 N.

1.2. Sulfuric acid, 0.02 N.

1.3. Methyl orange indicator.

#### 2. Procedure

2.1. Non-carbonate hardness. Measure 200 ml. of the sample and 200 ml. of distilled water into two 500 ml. Pyrex or similar glass Erlenmeyer flasks. Mix thoroughly. Treat the contents of each flask in the following manner: Boil 15 minutes to expel free carbon dioxide. Add 25 ml. of soda reagent. Boil 10 minutes, cool, rinse into a 200 ml. graduated flask, and dilute to 200 ml. with boiled distilled water. Filter, rejecting the first 50 ml. and titrate 50 ml. of each filtrate with 0.02 N  $\text{H}_2\text{SO}_4$  in the presence of methyl orange.

Water naturally containing bicarbonate and carbonate in excess of calcium and magnesium requires a larger amount of acid to neutralize the sample after it has been treated than is required to neutralize the volume of soda reagent originally added.

If more than one-half of the added soda reagent is neutralized, repeat with a smaller volume of sample diluted to 200 ml. with distilled water; otherwise low values for non-carbonate hardness will be found. The calculation is changed in accordance with the volume of sample taken.

Non-carbonate Hardness as ppm.  $\text{CaCO}_3$  =  $[(\text{ml. H}_2\text{SO}_4 \text{ used for distilled water} - \text{ml. H}_2\text{SO}_4 \text{ used for sample}) \times 1000] \div \text{ml. sample}$ .

**2.2 Total hardness.** Add standard sulfuric acid to 200 ml. of the sample until the alkalinity is neutralized (Part I, Sec. 13, page 31). Then follow the non-carbonate hardness method. This gives fairly satisfactory estimates of total hardness of hard waters.

Total Hardness as ppm.  $\text{CaCO}_3$  =  $[(\text{ml. H}_2\text{SO}_4 \text{ used for distilled water} - \text{ml. H}_2\text{SO}_4 \text{ used for sample}) \times 1000] \div \text{ml. sample}$ .

## BIBLIOGRAPHY

- CLARK, T. The examination of water for towns for its hardness and for the incrustation it deposits on boiling. *Chem. Gaz.*, 5, 100 (1847).
- HEHNER, O. Estimation of hardness without soap solution. *Analyst*, 8, 77 (1883).
- PFEIFER, J. Critical studies on the examination and purification of boiler feed waters. *Z. angew. Chem.*, 15, 193 (1902).
- PROCTOR, H. R. Some recent methods of technical water analysis. *J. Soc. Chem. Ind.*, 23, 8 (1904).
- BLACHER, C., GRUNBERG, P., AND KISSA, M. Use of potassium palmitate in water analysis. *Chem. Stg.*, 37, 56-8 (1913).
- SUTER, M. The potassium palmitate test for hardness. *J. Amer. W. W. Assn.*, 29, 1001-9 (1937).
- SHEEN, R. T., AND NOLL, C. A. Determination of hardness in water by direct titration. *Proc. A. S. T. M.*, 37, II, 607 (1937).
- BREASEALE, E. L., AND GREENE, R. A. The determination of calcium and magnesium by soap titration. *J. Amer. W. W. Assn.*, 30, 1040 (1938).
- BROWN, K. W., AND VILLARUZ, P. A. Improvement in the soap-solution method for determining hardness of water. *J. Amer. W. W. Assn.*, 30, 1021 (1938).
- LANGLELIER, W. F. A motorized apparatus for the rapid determination of calcium and magnesium in water. *J. Amer. W. W. Assn.*, 32, 279 (1940).
- POLSKY, J. W., AND FEDDERN, E. C. Determination of calcium in the presence of magnesium by standard soap solution. A rapid titration method. *Ind. Eng. Chem., Anal. Ed.*, 14, 644 (1942).

## 11. pH Value

### A. ELECTROMETRIC—GLASS ELECTRODE

pH is defined as the negative logarithm of the hydrogen-ion normality.

$$\text{pH} = -\log \text{normality (H}^+)$$

Since the pH is obtained from electromotive force measurements of the sample, it is impossible to calculate the hydrogen-ion normality from the pH due to the presence of liquid-junction potentials, which cannot be eliminated. For most industrial practices, however, the pH data are accepted and interpreted as such without trying to go beyond them to the hydrogen-ion concentration. The relation of pH value to hydrogen-ion in moles per liter is given in the following table.

The hydrogen electrode offers little use for practical application; nevertheless, it is of the utmost theoretical importance because it gives the standard pH values to which all other methods must be referred and by which all standard solutions, new types of electrodes, and new pH methods must be checked.

The glass electrode is the most accurate of the practical methods for determining the pH of a wide variety of

pH	Hydrogen ion mols per liter
n.00	$1.00 \times 10^{-n}$
n.05	$8.91 \times 10^{-(n+1)}$
n.10	7.94
n.15	7.07
n.20	6.31
n.25	5.62
n.30	5.01
n.35	4.46
n.40	3.98
n.45	3.54
n.50	3.16
n.55	2.82
n.60	2.51
n.65	2.24
n.70	1.99
n.75	1.78
n.80	1.58
n.85	1.41
n.90	1.26
n.95	1.12
(n + 1) .00	1.00

EXAMPLE: Find the H-ion in mols per liter corresponding to pH = 6.55  $n = 6$   $n + 1 = 7$ . From table H-ion concentration =  $2.82 \times 10^{-7}$ .

solutions. The limitations of the glass electrode, fragile glass and high electrical resistance, have been overcome by commercial apparatus readily available. Extraneous potentials, called asymmetry potentials, existing across the walls of glass electrodes can be corrected for by the use of buffer solutions of known pH values. The deviation of the glass electrode from accurate functioning as a hydrogen electrode above a pH of 10 can be diminished by the use of special glass for that purpose or by calibration of the electrode in alkaline buffer solutions of known pH and varying sodium ion concentrations (assuming sodium hydroxide is the base present).

Because of the effect of temperature on the measurement of pH, the pH electrometer used should contain temperature adjustments or a compensator which automatically allows for the proper conversion of electro-

motive force to pH at the temperature of the sample; otherwise, the value obtained can only be correct at one temperature.

The saturated calomel electrode is almost universally used as the reference electrode for pH measurement.

### 1. Sampling

If the sample being tested is poorly buffered as, e.g., condensate, the pH of the sample should be obtained without exposure of the sample to the atmosphere.

### 2. Apparatus

2.1. Glass electrode.

2.2. Saturated calomel electrode.

2.3. Buffer solutions pH 4.0, 7.0, 9.0.

2.4. pH electrometer.

It is recommended that the apparatus required be purchased from reputable manufacturers.

### 3. Standardization

The glass electrode assembly is standardized by placing it in a buffer solution of known pH and adjusting the electrometer to give that value by making the necessary asymmetry potential and temperature adjustments.

### 4. Procedure

Standardize the pH electrometer. In the case of unbuffered solutions, measure the pH of the sample in a closed system through which the solution passes. Allow the system to come to equilibrium. In the case of buffered solutions, the pH may be obtained on open samples. Enough tests should be taken so that the pH



value obtained remains constant. Keep the electrodes in distilled water when not in use. If the solution is hot or the pH is over 10, special glass electrodes should be used and the assembly should be standardized under conditions of temperature and concentration corresponding as closely as possible to the unknown.

## B. COLORIMETRIC

Colorimetric methods depend on the use of an indicator whose color in solution is characteristic of the pH value of the solution. If the solutions being tested are poorly buffered, as condensate or distilled water, special colorimetric technique is necessary.

For the reagents and procedure for the preparation of standard buffers, reference should be made to the Appendix I, Sec. 3, page 211. Any buffers so prepared should be checked by electrometric methods, e.g. the hydrogen or glass electrode.

### 1. Apparatus and Reagents

1.1. pH color comparator, complete with tubes.

1.2. Stock solutions of indicators. Weigh out carefully 0.1 g. of each indicator and grind, preferably in an agate mortar, with the volume of 0.05 N NaOH given in Table 7. When

solution is complete, make up to a volume of 25 ml. This is the stock solution; for use, dilute as directed below.

1.3. Test solution of indicators. Dilute 1 ml. of stock solution with 19 ml. of distilled water, with the exception of brom thymol blue and brom cresol purple; in these cases 1 ml. of stock solution should be diluted with 9 ml. of water.

1.4. Sodium hydroxide, approximately 0.05 N. Dissolve 2 g. of NaOH in distilled water and make up to 1 liter.

### 2. Procedure

Directions for making the determination of pH will be found with the particular pH color comparator being used. The exact amount of indicator to be used in the test will depend on the indicator and the size of the sample.

## BIBLIOGRAPHY

- SÖRENSEN, S. P. L., AND PALITZCH, S. The measurement of the hydrogen ion concentration of sea water. *Biochem. Z.*, **24**, 387 (1910).  
CLARK, WM. M., AND LUBS, H. A. The colorimetric determination of hydrogen ion concentration and its applications in bacteriology. *J. Bact.*, **2**, 109, 191 (1917).  
ACREE, S. F., MEACHEM, M. R., AND HOPFIELD, J. H. Preliminary note of the use of some mixed buffer materials for regulating the hydrogen ion concentrations of

TABLE 7.—INDICATORS, AND THE VOLUME OF 0.05 N SODIUM HYDROXIDE NECESSARY TO EFFECT SOLUTION OF 0.1 GRAM

Chemical name	Common name	pH range	Milliliters 0.05 N NaOH
Di bromo ortho cresol sulfonphthalein	brom cresol purple	5.2-6.8	3.7
Di bromo thymol sulfonphthalein	brom thymol blue	6.0-7.6	3.2
Phenol sulfonphthalein	phenol red	6.8-8.4	5.7
Cresol sulfonphthalein	cresol red	7.2-8.8	5.3
Thymol sulfonphthalein	thymol blue	8.0-9.6	4.3



culture media and of standard buffer solution. *J. Bact.*, **5**, 491 (1920).

GILLESPIE, L. J. Colorimetric determination of titration curves without buffer mixtures. *J. Am. Chem. Soc.*, **42**, 742 (1920).

CLARK, WM. M. *The Determination of Hydrogen Ions*. 3rd Ed. Williams & Wilkins, Baltimore (1928).

DOLE, M. *The Glass Electrode*, J. Wiley & Sons, New York (1941).

## 12. Acidity

### A. VOLUMETRIC

The acidity of a water is usually caused by the presence of uncombined carbon dioxide, mineral acids, and salts of strong acids and weak bases. The amount of acidity is determined by titration with a standard solution of a strong base to a definite equivalence point as given by indicator solutions. Phenolphthalein, or its equivalent, is used to obtain this equivalence point.

### 1. Reagents

1.1. Sodium hydroxide, 0.02 N, carbonate-free.

1.2. Phenolphthalein indicator solution.

1.3. Methyl orange indicator solution.

### 2. Procedure

2.1. Total acidity. Add 10 drops of phenolphthalein indicator to a suitable size sample, 50 or 100 ml. if possible, in a white porcelain casserole or an Erlenmeyer flask over a white surface. Titrate with 0.02 N NaOH until a pink color just appears.

Total Acidity as ppm.  $\text{CaCO}_3 = [\text{ml. of NaOH soln.} \times 1000] \div \text{ml. sample.}$

2.2. Mineral acid acidity. Add 2 drops of methyl orange indicator to a suitable size sample, 50 or 100 ml. if

possible, in a white porcelain casserole or an Erlenmeyer flask over a white surface. Titrate with 0.02 N NaOH until the color changes from pink to yellow.

Total Mineral Acid Acidity as ppm.  $\text{CaCO}_3 = [\text{ml. of NaOH soln.} \times 1000] \div \text{ml. sample.}$

## BIBLIOGRAPHY

ELLMs, J. W. AND BENEKER, J. C. The estimation of carbonic acid in water. *J. Am. Chem. Soc.*, **23**, 405 (1901).

MOREY, G. W. Benzoic acid as an acidimetric standard. Bureau of Standards, *Sci. Papers*, **183**, *Bull. 8*, 643 (1913).

HANDY, J. O. The determination of acidity and alkalinity. *Proc. Eng. Soc. West. Pa.*, **19**, 705 (1903).

JOHNSTON, J. The determination of carbonic acid, combined and free, in solution, particularly in natural waters. *J. Am. Chem. Soc.*, **38**, 947 (1916).

KOLTHOFF, I. M. Titration of carbonic acid and its salts. *Chem. Weekblad*, **14**, 780 (1917).

SCALES, F. M. Substitute for phenolphthalein and methyl orange in the titration of fixed and half-bound carbon dioxide. *Science*, n. s., **15**, 214 (1920).

SELVIG, W. A. AND RATCLIFF, W. C. The nature of acid waters from coal mines and the determination of acidity. *Ind. Eng. Chem.*, **14**, 125 (1922).

## 13. Alkalinity

### A. VOLUMETRIC

The alkalinity of a water is usually caused by the presence of carbonates, bicarbonates, hydroxides, and less frequently by borates, silicates, and phosphates. The amount of alkalinity is determined by titration with a standard solution of strong acid to certain equivalence points as given by indicator solutions. Phenolphthalein, or its equivalent, and methyl orange, or its equivalent, are used to obtain these equivalence points.

## 1. Reagents

- 1.1. Sulfuric acid, 0.02 N.
- 1.2. Sodium hydroxide, 0.02 N.
- 1.3. Phenolphthalein indicator solution.
- 1.4. Methyl orange indicator solution.

## 2. Procedure

2.1. Phenolphthalein alkalinity. Add 4 drops (0.1 ml.) of phenolphthalein indicator to a suitable size sample, 50 to 100 ml. if possible, in a white porcelain casserole or an Erlenmeyer flask over a white surface. Titrate with 0.02 N  $\text{H}_2\text{SO}_4$  until the pink coloration just disappears.

2.2. Methyl orange alkalinity. Add 2 drops of methyl orange indicator to the solution in which the phenolphthalein alkalinity has been determined, or to a suitable size sample, 50 or 100 ml. if possible, in a white porcelain casserole or an Erlenmeyer flask over a white surface. Titrate with 0.02 N  $\text{H}_2\text{SO}_4$  until the color changes from yellow to pink.

Phenolphthalein Alkalinity as ppm.  $\text{CaCO}_3$   
 $= [\text{ml. of } \text{H}_2\text{SO}_4 \times 1000] \div \text{ml. sample.}$

Methyl Orange Alkalinity as ppm.  $\text{CaCO}_3$   
 $= [\text{total ml. of } \text{H}_2\text{SO}_4 \times 1000] \div \text{ml. sample.}$

Methyl Orange Alkalinity as ppm.  $\text{Na}_2\text{CO}_3$   
 $= [\text{total ml. of } \text{H}_2\text{SO}_4 \times 1060] \div \text{ml. sample.}$

(See Part I, Sec. 3, B, 2-3, page 7.)

## BIBLIOGRAPHY

- HEHNER, O. Estimation of hardness without soap solution. *Analyst*, 8, 77 (1883).  
 THOMSON, R. T. Use of litmus, methyl orange, phenacetolin and phenolphthalein as indicators. *Chem. News*, 47, 123 (1883).  
 ELLMS, J. W. A study of the relative value of laemoid, phenacetolin and erythrosine as indicators in the determination of the alkalinity of water by Hehner's method. *J. Am. Chem. Soc.*, 21, 359 (1899).

WASHBURN, E. W. The significance of the term alkalinity in water analysis and the determination of alkalinity by means of indicators. *Proc. Ill. Water Supply Assn.*, 2, 93 (1910).

GREENFIELD, R. E., AND BARTOW, E. Note of a new indicator in water analysis. *Trans. Ill. Acad. Science*, 12, 326 (1920).

McKINNEY, D. S. Interpretation of water analysis. *Ind. Eng. Chem., Anal. Ed.*, 3, 192 (1931).

STRAUB, F. G. Determination of alkalinity in boiler water. *Ind. Eng. Chem., Anal. Ed.*, 4, 290 (1932).

## 14. Carbon Dioxide

### A. TOTAL CARBON DIOXIDE BY EVOLUTION METHOD

An excess of acid liberates carbon dioxide from bicarbonates and carbonates. With the assistance of heating and carbon dioxide-free air for scrubbing, any uncombined carbon dioxide originally present in the solution, in addition to that liberated, is absorbed in a barium hydroxide solution whose acid equivalent is known. After the absorption of carbon dioxide, the residual barium hydroxide is titrated with a standard acid and the total carbon dioxide concentration calculated therefrom. Phosphates, silicates, and aluminates do not interfere and the method is considered to be accurate to within 5 per cent except in those instances where sulfides, sulfites, or salts of other volatile acids are present.

### 1. Apparatus

The apparatus shown in Fig. 3 consists of a box equipped with daylight bulbs and a ground glass panel for even illumination. Inside the box are fastened the stoppers connected to the burettes and air circulating system. A small hot plate may be swung

up to support the evolution flask (D). A pump (G) is provided to circulate air, which passes from the evolution flask into the absorption tube (E) through a fritted glass disc sealed in glass.

## 2. Reagents

2.1. Sulfuric acid solution, 0.1 N.

2.2. Hydrochloric acid solution, 0.02 N. Add 20 ml. of phenolphthalein solution per liter before standardizing.

2.3. Barium hydroxide solution, 0.02 N. The acid equivalent of this solution is determined by using the evolution apparatus. Measure a 50 ml. sample from burette (A) into the absorption flask (E) and install the flask in the apparatus shown in Fig. 3.

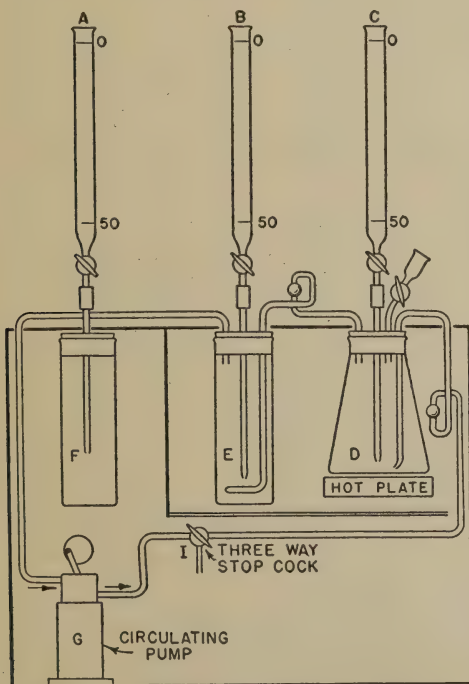


FIG. 3. APPARATUS ASSEMBLY FOR EVOLUTION METHOD FOR DETERMINING TOTAL CARBON DIOXIDE.

Place 200 ml. of carbon dioxide-free distilled water in the evolution flask (D), start the pump and circulate the gas for about 10 minutes. Titrate the barium hydroxide with 0.02 N HCl to the phenolphthalein end-point. The procedure outlined also serves to calibrate the apparatus against the air in the system.

2.4. Phenolphthalein indicator solution.

2.5. Methyl orange indicator solution.

## 3. Procedure

Measure 50 ml. of 0.02 N  $\text{Ba}(\text{OH})_2$  solution from burette (A) into the absorption flask in position (F), and place the flask in position (E).

Place a 200 ml. sample, containing not more than 100 ppm. total carbon dioxide, in the evolution flask. Add two drops of methyl orange indicator, and put the flask into position supported by the hot plate. Open the three-way cock to the atmosphere, start the pump, and when bubbles appear in the evolution flask, adjust the three-way cock to circulate gas. With the gas circulating (to stir the solution) add 0.1 N  $\text{H}_2\text{SO}_4$  to the sample to the methyl orange end point. Record the amount of acid used and then add 20 ml. in excess. Heat the solution only until it just reaches the boiling point and circulate the gas for about 15 minutes. Titrate the solution in the absorption flask (E) with 0.02 N HCl to the phenolphthalein end point.

## 4. Calculation

Total carbon dioxide may be calculated from the following formulas:



As ppm.  $\text{CO}_2 = (\text{ml. of acid equivalent to ml. Ba(OH)}_2 \text{ soln. used} - \text{ml. of 0.02 N HCl used in titration of sample}) \times 2.20$ .

As ppm.  $\text{HCO}_3 = (\text{ml. of acid equivalent to ml. Ba(OH)}_2 \text{ soln. used} - \text{ml. of 0.02 N HCl used in titration of sample}) \times 3.05$ .

As ppm.  $\text{CO}_3 = (\text{ml. of acid equivalent to ml. Ba(OH)}_2 \text{ soln. used} - \text{ml. of 0.02 N HCl used in titration of sample}) \times 3.00$ .

## B. FREE OR UNCOMBINED CARBON DIOXIDE BY CALCULATION

Free or uncombined carbon dioxide may be determined by calculation from either total carbon dioxide and pH or from total alkalinity and pH.

### 1. Procedure

1.1. From total  $\text{CO}_2$  and pH. The concentration of free  $\text{CO}_2$  may be estimated from the total carbon dioxide (Part I, Sec. 14, A, page 34), the pH (Part I, Sec. 11, page 28) of the sample and the curve shown in Fig. 4.

Determine the pH of the sample. Determine the total carbon dioxide of the sample. Then

ppm. free  $\text{CO}_2 = \text{Total CO}_2 \text{ as ppm. CO}_2 \times \text{fractional activity due to CO}_2$ .

1.2. From alkalinity and pH. The concentration of free  $\text{CO}_2$  may be estimated from the total alkalinity (Part I, Sec. 13, page 31), and the pH (Part I, Sec. 11, page 28) of the sample.

Determine the pH of the sample by the method described (Part I, Sec. 11, page 28). ( $\text{pH} = \log 1/[\text{H}^+]$ .) Determine the total alkalinity of the sample by the method described (Part I, Sec. 13, page 31).

Calculate the free  $\text{CO}_2$  from the following equation:

ppm. free  $\text{CO}_2 = 9.70 \times 10^{10}(\text{H}^+)$

$$\times \frac{\left[ \frac{\text{Alkalinity}}{50,000} + (\text{H}^+) - \frac{10^{-14}}{[\text{H}^+]} \right]}{1 + \frac{11.22 \times 10^{-11}}{(\text{H}^+)}}$$

The solution of this equation for various ppm. values of total alkalinity and pH may be obtained from Table 8.

## C. FREE OR UNCOMBINED CARBON DIOXIDE BY TITRATION

Sodium hydroxide combines with free carbon dioxide to form the bicarbonate ion. Completion of this reaction is indicated by the color change of phenolphthalein indicator.

### 1. Reagents

1.1. Sodium hydroxide, 0.02 N, carbonate-free.

1.2. Phenolphthalein indicator solution.

### 2. Procedure

Add 10 drops of phenolphthalein indicator solution to a suitable size sample, 100 ml. or greater if possible, in a glass stoppered volumetric flask over a white surface. Titrate with 0.02 N NaOH until a pink color appears.

ppm.  $\text{CO}_2 = [\text{ml. of NaOH} \times 880] \div \text{ml. sample}$ .

## BIBLIOGRAPHY

- ELLMs, J. W., AND BENEKER, J. C. The estimation of carbonic acid in water. *J. Am. Chem. Soc.*, 23, 405 (1901).
- HANDY, J. O. The determination of acidity and alkalinity. *Proc. Eng. Soc. West. Pa.*, 19, 705 (1903).

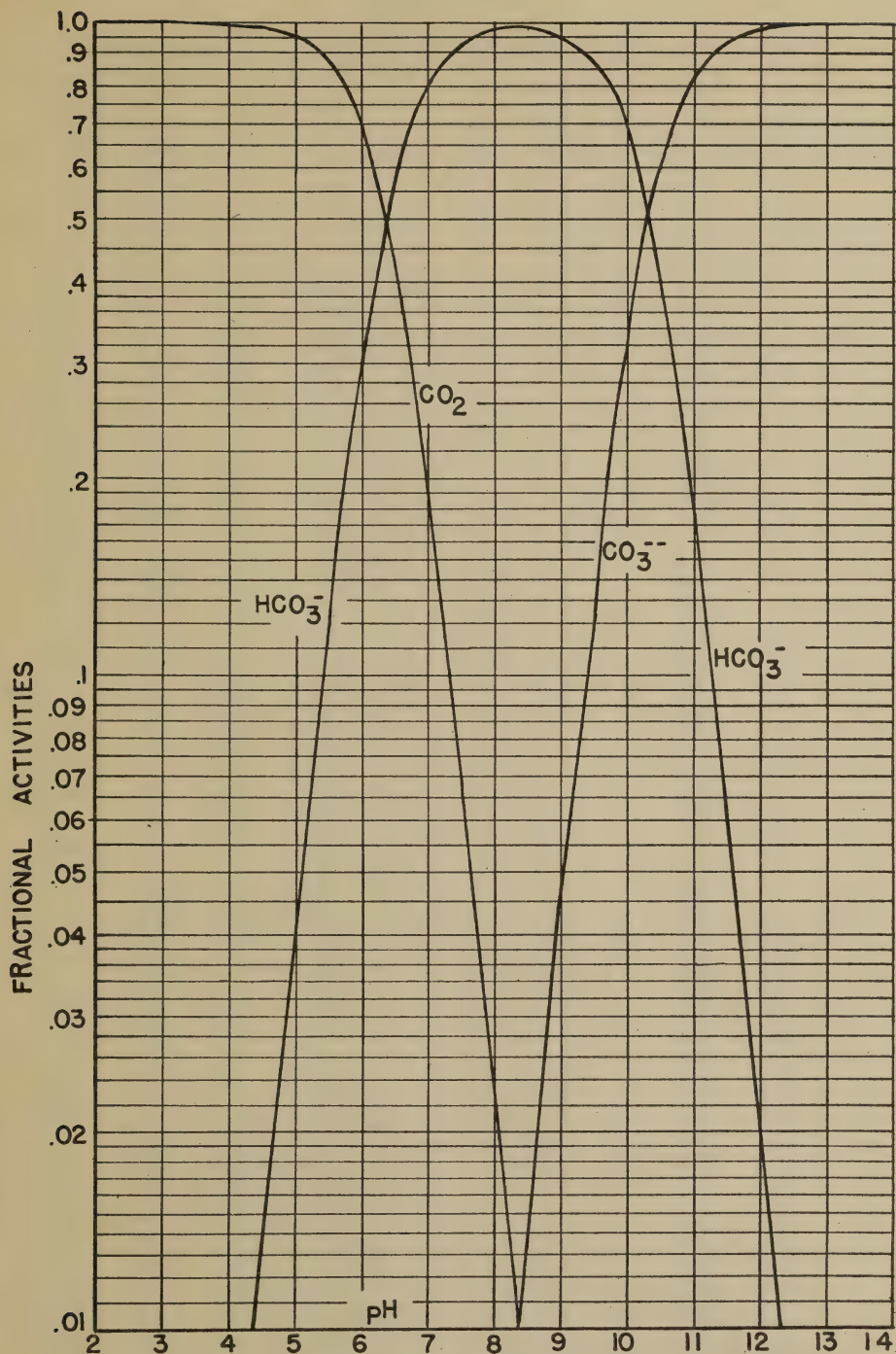


FIG. 4. GRAPH SHOWING RELATION OF pH AND CARBON DIOXIDE, CARBONATE AND BICARBONATE IONS AT DIFFERENT FRACTIONAL ACTIVITIES.  
(From chart submitted by M. C. Schwartz.)

TABLE 8.—RELATION BETWEEN pH AND FRACTIONAL ACTIVITIES OF ALKALINITY AND ACIDITY CONSTITUENTS\*

pH	Total Alk.	CO <sub>2</sub>	HCO <sub>3</sub> <sup>-</sup>	CO <sub>3</sub> <sup>-</sup>	pH	Total Alk.	HCO <sub>3</sub> <sup>-</sup>	CO <sub>3</sub> <sup>-</sup>	OH <sup>-</sup>
5.0	0	9.7	.5		8.4	100	97.3	2.8	
	2	48.5	2.5			200	194.5	5.5	
	100		100.5		8.6	100	95.5	4.3	
5.2	0	4.9				150	143.0	6.3	
	5	66.2			8.8	50	46.5	3.3	
5.4	0	1.5				150	140.0	9.9	
	5	40.3			9.0	50	44.5	5.0	0.5
5.5	0	1.0				150	134.5	15.0	0.5
	10	61.8			9.1	50	43.4	6.0	0.6
5.6	0	0.6				150	131.0	18.4	0.6
	10	49.3			9.2	50	41.9	7.4	0.7
5.7	0	0.4				150	126.8	22.5	0.7
	10	38.9			9.3	50	40.1	9.0	0.9
5.8	0	0.2				150	121.9	27.2	0.9
	10	30.9			9.4	50	38.1	10.7	1.2
5.9	0	0.1				150	116.1	32.7	1.2
	20	49.0			9.5	50	35.7	12.7	1.6
6.0	10	19.5	10.05			150	109.5	38.9	1.6
	20	38.9	20.05		9.6	50	33.2	14.8	2.0
6.1	10	15.4				150	102.3	45.7	2.0
	30	46.3			9.7	50	30.4	17.1	2.5
6.2	10	12.3				150	94.3	53.2	2.5
	30	36.8			9.8	50	27.4	19.4	3.2
6.3	10	9.7				150	85.9	60.9	3.2
	30	29.2			9.9	50	24.4	21.7	3.9
6.4	10	7.7				150	77.2	68.9	3.9
	50	38.7			10.0	50	21.2	23.8	5.0
6.5	10	6.1				150	68.2	76.8	5.0
	100	61.4			10.1	50	18.1	25.6	6.3
6.6	10	4.9				150	59.5	84.2	6.3
	100	48.8			10.2	50	15.1	27.0	7.9
6.7	10	3.8				150	51.1	91.0	7.9
	100	38.7			10.3	50	12.3	27.7	10.0
6.8	10	3.1				150	43.1	96.9	10.0
	100	30.7			10.4	50	9.8	27.7	12.5
6.9	10	2.4				150	36.1	101.4	12.5
	100	24.4			10.5	50	7.5	26.7	15.8
7.0	50	9.7	50			150	29.6	104.6	15.8
	200	38.7	200		10.6	50	5.5	24.6	19.9
7.1	50	7.7				150	23.8	106.3	19.9
	200	30.8			10.7	50	3.8	21.2	25.0
7.2	50	6.1				150	18.8	106.2	25.0
	200	24.5			10.8	50	2.3	16.2	31.5
7.3	50	4.9				150	14.6	103.9	31.5
	200	19.4			10.9	50	1.0	9.2	39.8
7.4	50	3.9				150	11.2	99.0	39.8
	200	15.6			11.0	50	0	0	50.0
7.5	50	3.1				200	12.3	137.7	50.0
	200	12.2			11.1	100	2.4	34.6	63.0
7.6	50	2.4				200	9.0	128.0	63.0
	200	9.7			11.2	100	1.1	19.6	79.3
7.7	50	1.9				200	6.4	114.3	79.3
	200	7.7			11.3	100	0	0	100.0
7.8	50	1.5				200	4.3	95.7	100.0
	200	6.1			11.4	150	0.8	23.6	125.6
7.9	50	1.2				200	2.6	71.8	125.6
	200	4.8			11.5	170	—	11.7	158.3
8.0	20		19.7			200	1.0	40.7	158.3
	100	1.9	98.9	1.1					
	200	3.8	198.0	2.2					
8.2	100	1.2							
	200	2.4							

\* Moore. *J. Amer. W. W. Assn.*, 31, 58 (1939).



JOHNSTON, J. The determination of carbonic acid, combined and free, in solution, particularly in natural waters. *J. Am. Chem. Soc.*, 38, 947 (1916).

KOLTHOFF, I. M. Titration of carbonic acid and its salts. *Chem. Weekblad*, 14, 780 (1917).

HALL, R. E., AND OTHERS. A physico-chemical study of scale formation and boiler water conditioning. *Carnegie Institute of Technology. Bulletin No. 24*, p. 162 (1927).

PARTRIDGE, E. P., AND SCHROEDER, W. C. Determination of hydroxide and carbonate in boiler waters. *Ind. Eng. Chem., Anal. Ed.*, 4, 3, 271; 274; 278 (1932).

SCHROEDER, W. C., AND PARTRIDGE, E. P. Determination of Carbonate, Phosphate and Hydroxide ions in Boiler Water. *A. S. M. E.* (1933).

PARTRIDGE, E. P., AND SCHROEDER, W. C. Progress report of Research Committee on Boiler Feed Water Studies. *A. S. M. E.* (1933).

TREADWELL, F. P., AND HALL, W. T. *Analytical Chemistry*. 8th Ed. Vol. II, p. 489, J. Wiley & Sons, New York (1935).

MOORE, E. W. Graphic determination of carbon dioxide and the three forms of alkalinity. *J. Amer. W. W. Assn.*, 31, 51 (1939).

McKINNEY, D. S. Calculation of corrections to conductivity measurements for dissolved gases. *Proc. A. S. T. M.*, 41, II, 1290 (1941).

AM. SOC. TEST. MTLs. Method for determination of total carbon dioxide and calculation of the carbonate and bicarbonate ions in industrial waters. *A. S. T. M. Method D 513-43*. *A. S. T. M. Supplement III*, 134 (1943).

## 15. Bicarbonate Ion

Methods for the determination of bicarbonate ion may be divided into two general classes (a) those involving an initial evolution of carbon dioxide and (b) those involving the direct titration of the sample. Methods falling under (a) are unaffected by the presence of other ions, except sulfides, sulfites, or other salts of volatile acids, and yield very satisfactory results. Methods falling under (b)

have been used with a fair degree of success where the bicarbonate ion concentrations are high and interfering ions are absent entirely or present only in small amount.

### A. BICARBONATE FROM TOTAL CARBON DIOXIDE BY CALCULATION

The concentration of bicarbonate (and carbonate ions and carbonic acid) ions in parts per million of  $\text{HCO}_3^-$  may be estimated from the total carbon dioxide (Part I, Sec. 14, page 32) the pH (Part I, Sec. 11, page 28) of the sample and the curve shown in Fig. 4 (Part I, Sec. 14, B, page 35).

#### 1. Procedure

Determine the pH of the sample. Determine the total carbon dioxide of the sample. Then,

$$\text{ppm. HCO}_3^- = \text{Total CO}_2 \text{ as ppm. HCO}_3^- \times \text{fractional activity due to HCO}_3^-.$$

### B. BICARBONATE FROM ALKALINITY BY CALCULATION

In an ideal system containing only the anions hydroxide, carbonate, and bicarbonate, the phenolphthalein and methyl orange alkalinity may be used to calculate the amounts of each ion. In practice, however, the ions may be calculated more accurately from an equation involving both the methyl orange alkalinity and the pH.

#### 1. Procedure

1.1. From alkalinity. Determine the phenolphthalein and methyl orange alkalinity (Part I, Sec. 13, page 31) of the sample. The value of the bicarbonate ion (and/or carbonate and hydroxide ions), expressed in

terms of calcium carbonate may be determined directly from the result of the titration by using the relations shown in Table 4 (Part I, Sec. 3, page 9).

1.2. From alkalinity and pH. Determine the pH of the sample ( $\text{pH} = \log 1/(\text{H}^+)$ ) as well as the methyl orange alkalinity, and calculate the bicarbonate ion value from the following equation:

ppm.  $\text{HCO}_3^-$  as  $\text{CaCO}_3 =$

$$\frac{50,000 \times \left[ \frac{\text{Alkalinity}}{50,000} + (\text{H}^+) - \frac{10^{-14}}{(\text{H}^+)} \right]}{1 + \frac{11.22 \times 10^{-11}}{(\text{H}^+)}}$$

The solution of this equation for various values of pH and alkalinity may be obtained from Table 8 (Part I, Sec. 14, page 36).

#### BIBLIOGRAPHY

- ELLMs, J. W., AND BENEKER, J. C. The estimation of carbonic acid in water. *J. Am. Chem. Soc.*, **23**, 405 (1901).
- HANDY, J. O. The determination of acidity and alkalinity. *Proc. Eng. Soc. West. Pa.*, **19**, 705 (1903).
- JOHNSTON, J. The determination of carbonic acid, combined and free, in solution, particularly in natural waters. *J. Am. Chem. Soc.*, **38**, 947 (1916).
- KOLTHOFF, I. M. Titration of carbonic acid and its salts. *Chem. Weekblad*, **14**, 780 (1917).
- HALL, R. E., AND OTHERS. A physico-chemical study of scale formation and boiler water conditioning. *Carnegie Institute of Technology. Bulletin No. 24*, p. 162 (1927).
- PARTRIDGE, E. P., AND SCHROEDER, W. C. Determination of hydroxide and carbonate in boiler waters. *Ind. Eng. Chem., Anal. Ed.*, **4**, 3, 271; 274; 278 (1932).
- SCHROEDER, W. C., AND PARTRIDGE, E. P. Determination of Carbonate, Phosphate and Hydroxide ions in Boiler Water. *A. S. M. E.* (1933).
- PARTRIDGE, E. P., AND SCHROEDER, W. C. Progress report of Research Committee on Boiler Feed Water Studies. *A. S. M. E.* (1933).
- TREADWELL, F. P., AND HALL, W. T. *Analytical Chemistry*. 8th Ed. Vol. II, p. 489, J. Wiley & Sons, New York (1935).
- MOORE, E. W. Graphic determination of carbon dioxide and the three forms of alkalinity. *J. Amer. W. W. Assn.*, **31**, 51 (1939).
- McKINNEY, D. S. Calculation of corrections to conductivity measurements for dissolved gases. *Proc. A. S. T. M.*, **41**, II, 1290 (1941).
- AM. Soc. TEST. MTLs. Method for determination of total carbon dioxide and calculation of the carbonate and bicarbonate ions in industrial waters. *A. S. T. M. Method D. 513-43*. *A. S. T. M. Supplement III*, 134 (1943).

#### 16. Carbonate Ion

Methods for the determination of the carbonate ion may be divided into two general classes: (a) those involving an initial evolution of carbon dioxide, and (b) those involving the direct titration of the sample. Methods falling under (a) are unaffected by the presence of other ions, except sulfides, sulfites, or other salts of volatile acids, and yield very satisfactory results. Methods falling under (b) have been used with a fair degree of success where the carbonate ion concentrations are high and interfering ions are absent entirely or present only in small amount.

#### A. CARBONATE FROM TOTAL CARBON DIOXIDE BY CALCULATION

The concentration of carbonate (and bicarbonate and carbonic acid) ions in parts per million of  $\text{CO}_2$  may be estimated from the total carbon dioxide (Part I, Sec. 14, page 32), the pH (Part I, Sec. 11, page 28) of the sample and the curve shown in Fig. 4 (Part I, Sec. 14, B, page 35).

### 1. Procedure

Determine the pH of the sample. Determine the total carbon dioxide of the sample. Calculate the concentration of  $\text{CO}_3^{=}$  from the following equation:

ppm.  $\text{CO}_3^{=}$  = Total  $\text{CO}_2$  as ppm.  $\text{CO}_3^{=}$   $\times$  fractional activity due to  $\text{CO}_3^{=}$ .

### B. BARIUM CHLORIDE METHOD

In order to determine the carbonate ion concentration of a sample by this procedure, the acid equivalent of the hydroxide ion concentration must be determined by the barium chloride method. In addition, a titration for the combined hydroxide and carbonate ions is made. From the values obtained, the carbonate ion concentration is calculated.

#### 1. Reagents

1.1. Hydrochloric acid, 0.02 N.

1.2. Barium chloride solution. Dissolve 100 g. of  $\text{BaCl}_2 \cdot \text{H}_2\text{O}$  in one liter of distilled water.

1.3. Phenolphthalein indicator solution.

#### 2. Procedure

Measure 100 ml. of water into a 250 ml. white porcelain casserole and add 2 drops of phenolphthalein indicator solution. Titrate the sample to the phenolphthalein end-point with 0.02 N HCl and record the volume of acid used as  $V_1$ .

Measure a second 100 ml. sample into a casserole. Add a slight excess of barium chloride solution and 2 drops of phenolphthalein indicator solution. Titrate the sample to the phenolphthalein end-point with 0.02

N HCl and record the volume of acid used as  $V_2$ .

Calculate the carbonate ion concentration from the following equation:

ppm.  $\text{CO}_3^{=}$  =  $[(V_1 - V_2) \times 600] \div \text{ml. of sample.}$

### C. CARBONATE FROM ALKALINITY BY CALCULATION

In an ideal system, containing only the anions hydroxide, carbonate, and bicarbonate, the phenolphthalein and methyl orange alkalinity may be used to calculate the amounts of each ion. In practice, however, the ions may be calculated more accurately from an equation involving both methyl orange alkalinity and the pH.

#### 1. Procedure

1.1. From alkalinity determine the phenolphthalein and methyl orange alkalinity (Part I, Sec. 13, page 31) of the sample. The value of the carbonate ion (and/or bicarbonate and hydroxide-ions) expressed in terms of calcium carbonate may be determined directly from the results of the titration by using the relations shown in Table 4 (Part I, Sec. 3, page 9).

1.2. From alkalinity and pH. Determine the pH (Part I, Sec. 11, page 28) of the sample as well as the methyl orange alkalinity, and calculate the carbonate ion value from the following equation:

ppm.  $\text{CO}_3^{=}$  as  $\text{CaCO}_3$  =

$$\frac{5.61 \times 10^{-6}}{(\text{H}^+)} \times \frac{\left[ \frac{\text{Alkalinity}}{50,000} + (\text{H}^+) - \frac{10^{-14}}{(\text{H}^+)} \right]}{1 + \frac{11.22 \times 10^{-11}}{(\text{H}^+)}}$$

The solution of this equation for various ppm. values of alkalinity and



pH may be obtained from Table 8 (Part I, Sec. 14, page 36).

## BIBLIOGRAPHY

- ELLMs, J. W., AND BENEKER, J. C. The estimation of carbonic acid in water. *J. Am. Chem. Soc.*, **23**, 405 (1901).
- HANDY, J. O. The determination of acidity and alkalinity. *Proc. Eng. Soc. West. Pa.*, **19**, 705 (1903).
- JOHNSTON, J. The determination of carbonic acid, combined and free, in solution, particularly in natural waters. *J. Am. Chem. Soc.*, **38**, 947 (1916).
- KOLTHOFF, I. M. Titration of carbonic acid and its salts. *Chem. Weekblad*, **14**, 780 (1917).
- HALL, R. E., AND OTHERS. A physico-chemical study of scale formation and boiler water conditioning. *Carnegie Institute of Technology. Bulletin No. 24*, p. 162 (1927).
- PARTRIDGE, E. P., AND SCHROEDER, W. C. Determination of hydroxide and carbonate in boiler waters. *Ind. Eng. Chem., Anal. Ed.*, **4**, 3, 271; 274; 278 (1932).
- SCHROEDER, W. C., AND PARTRIDGE, E. P. Determination of Carbonate, Phosphate and Hydroxide ions in Boiler Water. *A. S. M. E.* (1933).
- PARTRIDGE, E. P., AND SCHROEDER, W. C. Progress report of Research Committee on Boiler Feed Water Studies. *A. S. M. E.* (1933).
- TREADWELL, F. P., AND HALL, W. T. *Analytical Chemistry*. 8th Ed. Vol. II, p. 489, J. Wiley & Sons, New York (1935).
- MOORE, E. W. Graphic determination of carbon dioxide and the three forms of alkalinity. *J. Amer. W. W. Assn.*, **31**, 51 (1939).
- MCKINNEY, D. S. Calculation of corrections to conductivity measurements for dissolved gases. *Proc. A. S. T. M.*, **41**, II, 1290 (1941).
- AM. SOC. TEST. MTLs. Method for determination of total carbon dioxide and calculation of the carbonate and bicarbonate ions in industrial waters. *A. S. T. M. Method D. 513-43*. *A. S. T. M. Supplement III*, 134 (1943).

## 17. Hydroxide

### A. STRONTIUM CHLORIDE METHOD

Strontium chloride in controlled dosage precipitates carbonates and

phosphates from solution as strontium carbonate and strontium phosphate. The hydroxide ion is then titrated using phenolphthalein indicator. When only hydroxide and carbonate ions are present the method yields an accuracy slightly less than 1 per cent. If the phosphate ion is present the accuracy lies between 1 and 2 per cent. Organic matter, silicates, and aluminates tend to cause indefinite inaccuracies, but if present in very low concentrations as compared with the hydroxide ions, the method may be used; if present in high concentration, the method is not recommended.

### 1. Reagents

1.1. Hydrochloric or sulfuric acid solution, 0.02 N.

1.2. Strontium chloride solution. Dissolve 4.5 g. of  $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$  in 1 liter of distilled water.

1.3. Phenolphthalein indicator solution.

1.4. Purple indicator solution, LaMotte, 1 per cent. The use of purple indicator is recommended when phosphates are present, since under these conditions the phenolphthalein endpoint at a pH value of 8.0 fades rapidly due to the re-solution of strontium phosphate. At a pH above 9.3 this does not occur so rapidly.

### 2. Procedure

Measure accurately 100 ml. of the sample containing not more than 150 ppm. of hydroxide ion into a 250 ml. Erlenmeyer flask. Use a smaller sample if the concentration of hydroxide is known to be higher than 150 ppm. and multiply the results by the cor-

rect factor to obtain the final hydroxide value. To this sample add quickly 1 ml. of the  $\text{SrCl}_2$  solution for each 10 ppm. of carbonate ion present, 1 ml. for each 10 ppm. of phosphate ion present, and 4 ml. excess.

If the magnitude of phosphate and carbonate concentrations in the sample is not known, it will be necessary to add sufficient strontium chloride solution to precipitate what is thought to be the maximum concentrations of these ions that could exist in the water. The excess strontium will produce no interference but will increase the turbidity of the sample by precipitation of strontium sulfate.

Place a stopper very lightly in the flask. Heat to boiling with the stopper in place, but do not allow to boil for more than two or three seconds. Allow to stand hot for two or three minutes, then cool under water if desired. The stopper should be in the flask at all times to prevent carbon dioxide from entering.

If phosphate is absent add two or three drops of the phenolphthalein solution and titrate with the 0.02 N acid until the phenolphthalein is colorless. Swirl the sample steadily during the addition of the acid to prevent redissolving of the precipitate. If phosphate is present add 1 ml. of purple indicator solution and titrate to the end-point of this indicator with the 0.02 N acid. A comparison blank should be used if the operator is not familiar with the end-point of this indicator. Swirl the sample during the titration.

The concentration of the hydroxide ion in parts per million is calculated as follows:

ppm.  $\text{OH}^- = [\text{ml. acid} \times 340] \div \text{ml. of sample.}$

## B. BARIUM CHLORIDE METHOD

Barium chloride precipitates carbonates and sulfates from solution and permits a direct titration of the hydroxide ion providing no interfering substances are present, such as moderate amounts of silica, organic matter, and aluminates. The method is considered accurate to within 10 per cent.

### 1. Reagents

1.1. Hydrochloric acid, 0.02 N.

1.2. Barium chloride solution. Dissolve 100 g. of  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$  in one liter of distilled water.

1.3. Phenolphthalein indicator solution.

### 2. Procedure

To a 100 ml., or suitable size sample, add 5 ml. of  $\text{BaCl}_2$  solution to precipitate all of the carbonate and sulfate ions. (Water low in carbonate and/or sulfate will not produce a precipitate unless a small per cent of  $\text{Na}_2\text{CO}_3$  is added to the  $\text{BaCl}_2$  solution. The fine particles resulting act as nuclei for precipitation in the sample when the  $\text{BaCl}_2$  is added.) Add two or three drops of the phenolphthalein indicator solution and titrate to the end point (disappearance of color) with the standard HCl soln. Sulfuric acid is less desirable because of the production of barium sulfate and the possibility of occlusion of hydroxide in the precipitate.

ppm.  $\text{OH}^- = [\text{ml. acid} \times 340] \div \text{ml. sample.}$

## C. HYDROXIDE FROM ALKALINITY OR pH BY CALCULATION

In an ideal system containing only the anions hydroxide, carbonate, and bicarbonate, the phenolphthalein and methyl orange alkalinity can be used to calculate the amounts of each ion. In practice, however, the ions may be calculated more accurately from an equation involving both methyl orange alkalinity and the pH.

### 1. Procedure

1.1. From alkalinity. Determine the phenolphthalein and methyl orange alkalinity (Part I, Sec. 13, page 31) of the sample. The value of the hydroxide ion (and/or carbonate and bicarbonate ions) expressed in terms of calcium carbonate may be determined directly from the results of the titration by using the relations shown in Table 4 (Part I, Sec. 3, page 9).

1.2. From pH. Determine the pH (Part I, Sec. 11, page 28) of the sample ( $\text{pH} = \log 1/[\text{H}^+]$ ) and calculate the hydroxide ion value from the following equation:

$$\text{OH}^- \text{ as ppm. CaCO}_3 = [5 \times 10^{-10}] \div \text{H}^+.$$

The solution of this equation for various ppm. values of alkalinity and pH may be secured from Table 8 (Part I, Sec. 14, page 36).

## BIBLIOGRAPHY

- HALL, R. E., AND OTHERS. A physico-chemical study of scale formation and boiler water conditioning. *Carnegie Institute of Technology, Bulletin No. 24*, p. 162 (1927).
- ANON. Power station chemistry. A report of the Prime Movers Committee, *Publication 14, N. E. L. A.* (1931).

PARTRIDGE, E. P., AND SCHROEDER, W. C. Determination of hydroxide and carbonate in boiler water. *Ind. Eng. Chem., Anal. Ed.*, 4, 3, 271; 274; 278 (1932).

SCHROEDER, W. C., AND PARTRIDGE, E. P. Determination of Carbonate, Phosphate, and Hydroxide ions in Boiler Water. *A. S. M. E.* (1933).

JOINT RESEARCH COMMITTEE ON BOILER FEEDWATER STUDIES. Subcommittee No. 8. *Report No. 2* (1932).

TREADWELL, F. D., AND HALL, W. T. *Analytical Chemistry*. 8th Ed. Vol. 2, p. 489. J. Wiley & Sons, New York (1935).

MOORE, E. W. Graphic determination of carbon dioxide and the three forms of alkalinity. *J. Amer. W. W. Assn.*, 31, 51 (1939).

AM. SOC. TEST. MTLs. Method for determination of the hydroxide ion in industrial waters. A. S. T. M. Method D. 514-41. *A. S. T. M.*, III, 810 (1942).

## 18. Oil

Oil may be extracted from aqueous solution by a variety of organic solvents. The solvents differ, however, in their ability to extract not only the oil but other substances which may also be present. Since the oil may be saponified by sodium hydroxide present in the sample, it is advantageous to add acid before extraction.

### A. EVAPORATION AND EXTRACTION

#### 1. Reagents

1.1. Diethyl ether.

1.2. Concentrated hydrochloric acid.

#### 2. Procedure

Add 1 ml. of concd. HCl to a suitable size sample and evaporate in a porcelain dish to less than 100 ml. During evaporation, the sample should be protected against contamination. Transfer the solution to a small Pyrex beaker taking care that all the oil is removed from the dish. Evaporate



the solution to dryness on a water bath. Cool and add diethyl ether to the residue. Filter the solution into a light evaporating dish or beaker, previously dried at 103° C. and weighed. Wash the filter paper with several portions of diethyl ether. Evaporate the filtrate, dry at 103° C., then weigh the residue. Determine the blank correction by following the procedure beginning with the washing of the filter paper and using the same total quantity of diethyl ether.

ppm. of Oil =  $\frac{(\text{wt. of residue from sample} - \text{wt. of residue from blank}) \times 1000}{\div \text{ml. sample.}}$

## B. WET EXTRACTION METHOD

### 1. Reagents

- 1.1. Diethyl ether.
- 1.2. Concentrated hydrochloric acid.

### 2. Procedure

Acidify a suitable size sample, placed in a clean separatory funnel, with 2 ml. of concd. HCl and shake. Extract the oil from the solution with a sufficient number of 25 ml. portions of ether. The ether layer is removed each time and the sample is again treated with a portion of ether. The solvent extractions are collected in a light evaporating dish or beaker, previously dried and weighed. Evaporate the solution, dry at 103° C. and then weigh the residue. Determine the blank correction by obtaining the residue on evaporation of a portion of ether equal to that used on the sample.

ppm. of Oil =  $\frac{(\text{wt. of residue from sample} - \text{wt. of residue from blank}) \times 1000}{\div \text{ml. sample.}}$

## BIBLIOGRAPHY

GRIFFIN, R. C. *Technical Methods of Analysis*, 2nd Ed., p. 702, McGraw Hill, New York (1927).

## 19. Silica

### A. GRAVIMETRIC

Hydrochloric acid decomposes soluble silicates forming silicic acids which are precipitated as partially dehydrated silica by complete evaporation and heating. A double evaporation is necessary for complete removal of silica. Ignition completes the dehydration of silica which is then volatilized as silicon tetrafluoride.

Perchloric acid may be used in place of hydrochloric acid. The dehydration of silica is accomplished in less time than with hydrochloric acid; a single evaporation to fumes with perchloric acid, however, does not permit recovery of all the silica.

### 1. Sampling

Collect the sample in a hard rubber or a Pyrex bottle. In the latter case, keep the sample as cool as possible while in contact with glass.

### 2. Reagents

- 2.1. Hydrochloric acid, 1:1.
- 2.2. Hydrochloric acid, 1:50.
- 2.3. Sulfuric acid, 1:1.
- 2.4. Hydrofluoric acid, concentrated, 48 per cent. Check the non-volatile content of the 1:1 H<sub>2</sub>SO<sub>4</sub> and the HF by adding a few drops of the former and 10 ml. of the latter to a platinum crucible, evaporating the acids to dryness, igniting at a high temperature (1200° C. optimum temperature) for 15 minutes and weighing the residue.

2.5. Perchloric acid, 72 per cent, silica free.

### 3. Procedure

3.1. Hydrochloric acid method. To a clear sample containing at least 10 mg. silica, add 5 ml. of 1:1 HCl. Evaporate the solution in an acid cured, glazed porcelain evaporating dish (platinum is preferred when available) on a water bath, adding 1:1 HCl, in several portions, making a total of 15 ml.

After evaporation, dry the residue at 110° C. for 1 hour. Add 5 ml. of 1:1 HCl to the residue in the dish, warm, then add 50 ml. of distilled water. Filter the warm solution, decanting as much of the solution as possible.

Wash the residue with 1:50 HCl, and with as small an amount of distilled water as possible, until the washings are chloride-free. Save the filter paper and residue.

Evaporate the filtrate and washings to dryness in the original dish. Dry the residue at 110° C. for 30 minutes. Dissolve the residue and filter through a separate filter paper, as described for the first evaporation, taking especial care to effect complete removal of the silica to the filter paper.

Dry the two filter papers and residues and carefully ignite to constant weight (1200° C. optimum temperature) in a covered crucible (platinum preferably). Thoroughly moisten the residue in the crucible with distilled water.

Add a few drops of 1:1 H<sub>2</sub>SO<sub>4</sub> and then 10 ml. of HF. Evaporate the solution to dryness over an air bath. Ignite the residue (1200° C. optimum temperature) to constant weight.

ppm. SiO<sub>2</sub> = [1000 (mg. SiO<sub>2</sub> after ignition — mg. residue after HF)] ÷ ml. sample.

3.2. Perchloric acid \* method. To a clear sample containing at least 10 mg. silica, add 5 ml. of concd. HCl. Evaporate the solution in an acid cured, glazed porcelain evaporating dish (platinum preferred when available) to 100 ml. Add 15 ml. of HClO<sub>4</sub> to the sample, and continue evaporation on a hot plate under a fume hood until dense white fumes of perchloric acid appear. Continue the dehydration for 10 minutes; cool somewhat, then dilute with 50 ml. of distilled water and add 5 ml. of concd. HCl.

Boil for several minutes, filter and wash the residue with 10 small portions of hot distilled water to remove HClO<sub>4</sub>. Dry the filter paper and residue and ignite to constant weight in a covered crucible (platinum preferably).

ppm. SiO<sub>2</sub> = mg. SiO<sub>2</sub> × 1000 ÷ ml. sample.

### B. COLORIMETRIC METHOD—MOLYBDATE

Ammonium molybdate, in an acid solution of approximately pH 1.2, produces a yellowish green color with crystalloidal or non-colloidal silica and phosphate. The addition of oxalic acid destroys any color contributed by the phosphate. Tannins interfere.

#### 1. Sampling

Collect the sample in a hard rubber or a Pyrex bottle. In the latter case, keep the sample as cool as possible while in contact with glass.

\* The analyst should be familiar with the possible hazards involved before undertaking this method.

## 2. Apparatus

One of the following pieces of equipment is required.

2.1. Nessler tubes for visual color comparison.

2.2. Duboseq type colorimeter for visual color comparison.

2.3. Photoelectric filter photometer.

2.4. Photoelectric spectrophotometer.

## 3. Reagents

3.1. Ammonium molybdate solution. Dissolve 10 g. of  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4_2\text{HO}$  per 100 ml. of distilled water.

3.2. Hydrochloric acid, 1:1.

3.3. Oxalic acid solution. Dissolve 10 g. of  $\text{C}_2\text{H}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$  per 100 ml. of distilled water.

3.4. Sodium silicate standard solution. Fuse 3.0 g. of  $\text{Na}_2\text{CO}_3$  with 0.2000 g. of pure, dry silica in a platinum crucible. Dissolve in 200 ml. of distilled water. Dilute ten-fold if desirable.

The  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ ,  $\text{HCl}$ , and  $\text{C}_2\text{H}_2\text{O}_4$  solns. should be kept in Pyrex bottles; the  $\text{Na}_2\text{SiO}_3$  soln. should be kept in a hard rubber bottle.

## 4. Procedure

If phosphate is known to be absent, the addition of the  $\text{C}_2\text{H}_2\text{O}_4$  soln. may be omitted. To a 50 ml. clear sample, add in quick succession 2 ml. of  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$  soln. and 1 ml. of 1:1  $\text{HCl}$ . After five minutes, add 1.5 ml. of  $\text{C}_2\text{H}_2\text{O}_4$  soln. Compare colorimetrically against standards prepared simultaneously if using apparatus 2.1 or 2.2; or against a blank if using apparatus 2.3 or 2.4. A wavelength of 410 millimicrons is suitable for this

colorimetric determination. If apparatus 2.1 or 2.2 is used, a blank correction, if there is one, should be subtracted from the observed value.

## 5. Permanent Color Standards

Potassium chromate <sup>a</sup> ml.	Silica <sup>b</sup> ppm.
1.0	2
2.0	4
3.0	6
4.0	8
5.0	10
7.5	15
10.0	20

<sup>a</sup> 0.63 g. of  $\text{K}_2\text{CrO}_4$  per liter of distilled water. Dilute the volumes specified with 25 ml. of a 1 per cent solution of sodium tetraborate and enough water to make a total of 53 ml. (or 55 ml. if oxalic acid is used).

<sup>b</sup> When 50 ml. of sample are used together with the reagents indicated in the previously described procedure, to make a total volume of 53 ml. (or 55 ml. if oxalic acid is used).

## C. MODIFICATION—FOR TESTING CONDENSATE. MOLYBDATE AND REDUCTION WITH AMINO NAPHTHOL SULFONIC ACID

This procedure is suitable for testing condensate containing small amounts of silica and phosphate. One additional reagent to those in B above, is required.

### 1. Reagent

Amino naphthol sulfonic acid solution. Dissolve 0.5 g. of amino naphthol sulfonic acid in a solution of 30 g. of  $\text{NaHSO}_3$  in 200 ml. of distilled water, add 1 g. of  $\text{Na}_2\text{SO}_3$  and heat to assist solution. Prepare fresh every two weeks, and keep in a dark Pyrex bottle.

### 2. Procedure

One minute after the addition of oxalic acid (Part I, Sec. 19, B, 4, page 45), add 2 ml. of amino naphthol sulfonic acid solution. Compare colori-



metrically after two minutes against a blank using apparatus 2.3 or 2.4. A wavelength of 700 millimicrons is suitable for this colorimetric determination.

#### D. COLORIMETRIC METHOD—MOLYBDATE AND REDUCTION WITH SODIUM SULFITE

Ammonium molybdate, in an acid solution of pH 2.4–2.7, in the presence of a reducing agent as sodium sulfite, produces a blue color with crystalloidal or non-colloidal silica, suitable for colorimetric determination. The presence of phosphate and tannins offers no practical interference.

#### 1. Apparatus

One of the following pieces of equipment is required.

1.1. Duboseq type colorimeter for visual color comparison.

1.2. Photoelectric filter photometer.

1.3. Photoelectric spectrophotometer.

#### 2. Reagents

2.1. Hydrochloric acid, 0.248 N.

2.2. Ammonium molybdate solution. Dissolve 102 g. of  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$  in 1 liter of distilled water.

2.3. Sodium sulfite solution. Dissolve 170 g. of  $\text{Na}_2\text{SO}_3$  in 1 liter of distilled water.

#### 3. Procedure

To a maximum of 10 ml. of clear sample add, with mixing, 5 ml. of 0.248 N HCl and 5 ml. of  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$  soln. After 1 minute, add 10 ml. of  $\text{Na}_2\text{SO}_3$  soln. and mix. Com-

pare colorimetrically after 1 minute against standards prepared simultaneously if using apparatus 1.1; or against a blank if using apparatus 1.2 or 1.3. If apparatus 1.1 is used, a blank correction, if there is one, should be subtracted from the observed value.

A wavelength of 620 millimicrons is suitable for colorimetric determination. The temperature of the sample and reagents should be maintained within  $\pm 2.8^\circ \text{C}$ . of the solution temperature at the time of standardization.

#### BIBLIOGRAPHY

- ISAACS, L. Presence of silicon in tissues. A micro-method for the determination of silicon. *Bull. Soc. chim. biol.*, 6, 157 (1924).
- SWANK, H. W., AND MELLON, M. G. Colorimetric standards for silica. *Ind. Eng. Chem., Anal. Ed.*, 6, 348 (1934).
- KAHLER, H. L. Determination of soluble silica in water. A photometric method. *Ind. Eng. Chem., Anal. Ed.*, 13, 536 (1941).
- SCHWARTZ, M. C. Photometric determination of silica in the presence of phosphates. *Ind. Eng. Chem., Anal. Ed.*, 14, 893 (1942).
- STRAUB, F. G., AND GRABOWSKI, H. A. Photometric determination of silica in condensed steam in the presence of phosphates. *Ind. Eng. Chem., Anal. Ed.*, 16, 574 (1944).

#### 20. Arsenic

Arsenic is liberated as arsine by means of the Gutzeit method and then absorbed in sodium hypobromite solution. Ammonium molybdate, in acid solution in the presence of hydrazine sulfate as a reducing agent, produces a blue color with the oxidized arsenic suitable for colorimetric determination.

## 1. Apparatus

- 1.1. Gutzeit generator.
- 1.2. Absorption tube.
- 1.3. Nessler tubes.

## 2. Reagents

2.1. Sodium hypobromite solution. Add 20 ml. of 0.5 N NaOH soln. to a solution of 30 ml. of saturated bromine water and 30 ml. of distilled water.

2.2. Ammonium molybdate solution. Dissolve 25 g. of  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$  in 300 ml. of distilled water. Dilute 75 ml. of concd.  $\text{H}_2\text{SO}_4$  with distilled water to a volume of 200 ml. and add to the molybdate solution.

2.3. Hydrazine sulfate solution. Dilute 50 ml. of a saturated hydrazine sulfate solution with 50 ml. of distilled water.

2.4. Sulfuric acid, 2 N.

2.5. Sulfuric acid, 2:1 solution.

2.6. Standard arsenious oxide solution. Dissolve 0.300 g. of  $\text{As}_2\text{O}_3$  in 25 ml. of 10 per cent sodium hydroxide. Acidify with 1:6  $\text{H}_2\text{SO}_4$  and dilute to 1 liter with distilled water. One ml. of this solution contains 0.3 mg. of  $\text{As}_2\text{O}_3$ . Dilute this stock standard solution as required.

2.7. Zinc, 20–30 mesh, arsenic-free.

2.8. Stannous chloride solution. Dissolve 40 g. of arsenic-free  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$  in 1:3 HCl and dilute to 100 ml. with the same acid.

2.9. Potassium iodide solution. Dissolve 15 g. of KI in distilled water and dilute to 100 ml.

## 3. Procedure

Place a 25 ml. sample, or suitable aliquot, in a Gutzeit generator. Add

10 ml. of 2:1  $\text{H}_2\text{SO}_4$ , 5 ml. of KI soln. and 4 drops of  $\text{SnCl}_2$  soln. to the sample. Add 2–5 g. of zinc in the generator. Add 3 ml. of NaOBr to the absorption tube. Place the generator in a water bath at 20–25° C. for 1 to 1.5 hours; after complete evolution of arsine, wash out the absorption tube with six 2 ml. portions of distilled water.

Add to these washings, with mixing after each reagent addition, 5 ml. of 2 N  $\text{H}_2\text{SO}_4$ , 1 ml. of  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$  soln. and 1 ml. of hydrazine sulfate solution. Dilute to 25 ml. with distilled water and compare colorimetrically after 0.5 hour against standards prepared similarly and at the same time. Determine the arsenic content of reagents and distilled water by running a blank.

## BIBLIOGRAPHY

ASSOC. OFF. AGR. CHEM. *Methods of Analysis*, 5th Ed., pp. 390, 542 (1940).

JACOBS, M. B., AND NAGLER, J. Colorimetric microdetermination of arsenic. *Ind. Eng. Chem., Anal. Ed.*, 14, 442 (1942).

## 21. Copper

### A. COLORIMETRIC—CARBAMATE

In the presence of copper, sodium diethyldithiocarbamate produces a yellow color suitable for colorimetric estimation. The reagent is not specific for copper, but interfering substances are not common in water and their presence is easily detected. Zinc and lead produce a white turbidity. Iron interferes and a special procedure is presented for such a case. A suitable range for visual color comparison is 0.005 to 0.05 mg. of Cu.

### 1. Apparatus

One of the following pieces of equipment is required.

1.1. Nessler tubes for visual color comparison.

1.2. Duboseq type colorimeter for visual color comparison.

1.3. Photoelectric filter photometer.

1.4. Photoelectric spectrophotometer.

### 2. Reagents

2.1. Sodium diethyldithiocarbamate solution. Dissolve 1 g. of  $N(C_2H_5)_2 \cdot CS_2Na$  in 100 ml. of distilled water and keep in a bottle of dark glass protected from the light.

2.2. Copper-free distilled water for the preparation of reagents and dilution water.

2.3. Copper sulfate standard solution. Dissolve 0.393 g. of  $CuSO_4 \cdot 5H_2O$  in 1 liter of distilled water. Dilute 25 ml. to 250 ml. One ml. of the diluted solution contains 0.01 mg. of Cu.

2.4. Bipyridine solution. Dissolve 0.2 g. of 2,2'-bipyridine in 1 ml. of glacial acetic acid and dilute to 100 ml. with distilled water.

2.5. p-Hydroxyphenylglycine solution. Dissolve 0.5 g. of p-hydroxyphenylglycine in 100 ml. of 0.1 N sulfuric acid. Allow to settle and use the supernatant liquid. Prepare fresh before using.

2.6. Isoamyl acetate, or isoamyl alcohol, or carbon tetrachloride.

2.7. Sodium acetate solution. Dissolve 14 g. of  $NaC_2H_3O_2 \cdot 3H_2O$  in 100 ml. of distilled water.

2.8. Sulfuric acid, 1:6.

2.9. Ammonium hydroxide, 1:5.

### 3. Procedure

3.1. Iron-free sample. Place 50 ml. of the sample, diluted if necessary, in a 50 ml. Nessler tube. Quantities of copper exceeding 0.1 mg. give colors too deep for accurate visual comparison and in such cases an aliquot portion should be taken. Add 2 ml. of 1:5 ammonium hydroxide (if a precipitate forms, it will be advisable to use procedure 3.2). Add 1 ml. of sodium diethyldithiocarbamate solution. Compare colorimetrically against standards prepared simultaneously if using apparatus 1.1 or 1.2; or against a blank, if using 1.3 or 1.4. A wavelength of 440 millimicrons is suitable for this photometric comparison.

Should turbidity interfere with the comparison, extract the color by shaking with successive 3 to 5 ml. portions of  $CCl_4$  until no more color is extracted. In such cases, the comparison standards should be extracted in the same manner and with precisely the same volume of  $CCl_4$ . The extracted mixture from both the sample and standards should be transferred to tubes for observation.

3.2. Iron containing sample. Place 25 ml. of the sample in a Nessler tube. Add the following reagents, mixing after each addition: 1 ml. of 1:6  $H_2SO_4$ , 1 ml. of p-hydroxyphenylglycine solution, 2 ml. of bipyridine solution, and 5 ml. of  $NaC_2H_3O_2$  soln. Add 2 ml. of sodium diethyldithiocarbamate solution. Extract the color with 15 ml. of isoamyl acetate, isoamyl alcohol, or  $CCl_4$ . Compare colorimetrically against standards prepared simultaneously if using apparatus 1.1 or 1.2; or against a blank, if using



1.3 or 1.4. A wavelength of 440 millimicrons is suitable for this photometric comparison.

## BIBLIOGRAPHY

- CALLAN, THOMAS, AND HENDERSON, J. A. R. A new reagent for the colorimetric determination of minute amounts of copper. *Analyst*, 54, 650 (1929).
- HADDOCK, L. A., AND EVERS, N. The determination of minute amounts of copper in the presence of iron and certain other metals. *Analyst*, 57, 495 (1932).
- ANON. *Organic Reagents for Metals*, 88, 2nd Ed., Hopkin & Williams, Ltd., London (1934).
- GERBER, L., CLAASSEN, R. I., AND BORUFF, C. S. Photometric determination of copper and iron in distilled liquors. *Ind. Eng. Chem., Anal. Ed.*, 14, 364 (1942).
- STONE, I. Determination of traces of copper in wort, beer, and yeast. *Ind. Eng. Chem., Anal. Ed.*, 14, 479 (1942).

## 22. Lead

Lead is separated from solution as lead sulfide, isolated as lead sulfate, and finally determined colorimetrically as the sulfide.

### 1. Reagents

1.1. Lead nitrate standard solution. Dissolve 1.6 g. of  $\text{Pb}(\text{NO}_3)_2$  in 1 liter of distilled water. One ml. of this solution contains 1 mg. of lead. As a check, it is desirable to determine lead as the sulfate in a measured portion of this solution.

1.2. Ammonium chloride. Dissolve 25 g. of  $\text{NH}_4\text{Cl}$  in 100 ml. of distilled water.

1.3. Ammonium acetate. Dissolve 50 g. of  $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$  in 100 ml. of distilled water.

1.4. Hydrogen sulfide, saturated solution.

1.5. Acetic acid solution, 50 per cent.

1.6. Nitric acid solution, 1:9.

1.7. Ethyl alcohol solution, 1:1.

### 2. Procedure

Concentrate rapidly a suitable amount of the sample to be tested by boiling in a porcelain, or similar, dish, to a volume of about 30 ml. A large sample may be used if the amount of lead is very small. Add 10 or 15 ml. of  $\text{NH}_4\text{Cl}$  soln. to assist in the separation of the sulfides, then add a few drops of concd.  $\text{NH}_4\text{OH}$ , and saturate with  $\text{H}_2\text{S}$ . Allow to stand some time, preferably overnight, add a little more  $\text{NH}_4\text{OH}$  and  $\text{H}_2\text{S}$ , boil the contents of the dish a few minutes, and filter. The precipitate may consist of lead, zinc, copper and iron sulfides and the suspended organic matter. The soluble coloring matter is in the filtrate. Wash the precipitate a few times with hot water, place the precipitate and the filter paper in the original dish and boil with dil.  $\text{HNO}_3$ , rubbing down the sides of the dish, if necessary, to detach any adhering sulfide precipitate. After again filtering and washing several times with hot water, evaporate the filtrate and washings in the original dish to a bulk of 10 to 15 ml., cool, add 5 ml. of concd.  $\text{H}_2\text{SO}_4$  and heat until copious fumes of  $\text{H}_2\text{SO}_4$  are evolved.

Dilute the contents of the dish slightly with water, and treat them with 150 ml. of  $\text{C}_2\text{H}_5\text{OH}$  soln., in which the  $\text{PbSO}_4$  is insoluble. Allow to stand some time, preferably overnight, filter off the  $\text{PbSO}_4$  and wash it with  $\text{C}_2\text{H}_5\text{OH}$  soln.

Dissolve the precipitate of  $\text{PbSO}_4$  by boiling the filter containing it in  $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$  soln. in a porcelain dish.

Filter into a 50 ml. Nessler tube and wash the filter with boiling water, containing a little  $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$ . Divide this filtrate in halves and treat one-half with saturated  $\text{H}_2\text{S}$  water in order to get an approximation of the amount of lead present. To the other half, or an aliquot portion, if a large amount of lead is present, add a few drops of  $\text{CH}_3\text{COOH}$ , then an excess of saturated  $\text{H}_2\text{S}$  soln., and compare the color with that of standards made by treating known amounts of the standard lead solution with an equivalent amount of  $\text{CH}_3\text{COOH}$ ,  $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$  and  $\text{H}_2\text{S}$ .

#### BIBLIOGRAPHY

CLARK, H. W., AND FORBES, F. B. Methods for the determination of lead, tin, zinc and copper in drinking water. *Rept. State Bd. of Health, Mass.*, 577 (1898); 498 (1900).

### 23. Aluminum

#### A. GRAVIMETRIC

Ammonium hydroxide precipitates aluminum quantitatively in a solution whose pH is properly adjusted by the use of phenol red. Iron interferes.

##### 1. Reagents

1.1. Ammonium hydroxide 1:1.

1.2. Ammonium chloride solution. Dissolve 2 g. of  $\text{NH}_4\text{Cl}$  in 100 ml. of distilled water. Neutralize with 1:1  $\text{NH}_4\text{OH}$  to phenol red.

1.3. Phenol red indicator solution.

##### 2. Procedure

To a clear 100 to 200 ml. sample, add 4 to 5 g. of solid  $\text{NH}_4\text{Cl}$  and 0.5 ml. of phenol red solution. Heat the solution nearly to boiling and add 1:1  $\text{NH}_4\text{OH}$  until the color changes from yellow to orange. Boil for three min-

utes, filter and wash the precipitate with hot  $\text{NH}_4\text{Cl}$  solution. After drying and burning off the filter paper in a porcelain or platinum crucible, ignite the residue to constant weight at  $1200^\circ\text{C}$ .

ppm.  $\text{Al}^{+++} = [\text{mg. Al}_2\text{O}_3 \times 529.7] \div \text{ml. sample.}$

#### B. COLORIMETRIC

In dilute aluminum solutions, buffered to a pH of 6.3, the ammonium salt of aurin tricarboxylic acid (aluminon) produces a pink color suitable for colorimetric determination.

Chromium and iron will interfere, 0.1 mg. of Cr gives the same color as 0.0005 mg. of Al and 0.01 mg. of  $\text{Fe}^{+++}$  as much color as 0.005 mg. of Al. Interference by Cr will be seldom encountered in water analysis but Fe is often present. The literature does not mention Mn which is often present in waters and which interferes with the hematoxylin test.

##### 1. Apparatus

One of the following pieces of equipment is required.

1.1. Nessler tubes for visual color comparison.

1.2. Duboseq type colorimeter for visual color comparison.

1.3. Photoelectric filter photometer with a green filter (ca  $520\text{ m}\mu$ ).

1.4. Photoelectric spectrophotometer.

##### 2. Reagents

2.1. Aluminum-free distilled water for the preparation of reagents and dilution water.

2.2. Acetate buffer solution. Add 3 ml. of glacial acetic acid per 250

ml. total volume of 4 N ammonium acetate.

2.3. Aluminon solution. Dissolve 0.1 g. of the ammonium salt of aurintricarboxylic acid in 100 ml. of distilled water.

2.4. Aluminum standard solution. Prepare a solution of aluminum potassium sulfate ( $\text{Al}_2(\text{SO}_4)_3 \cdot \text{K}_2\text{SO}_4 \cdot 24\text{H}_2\text{O}$ ) containing 0.005 mg. Al per ml. by dissolving 8.7955 g. per liter and diluting the solution 1 to 100.

### 3. Procedure

To a 50 ml. of clear sample, add 2 ml. of acetate buffer solution and mix. Add 2 ml. of the aluminon solution and mix. Wait 15 minutes for full color development. Check the aluminum content of the reagents by carrying out the procedure on 50 ml. of aluminum-free water. Subtract the aluminum in the blank (if any), from the observed aluminum. Compare colorimetrically against standards prepared simultaneously if using Nessler tubes, or a Duboseq type colorimeter, or against a blank if using apparatus 1.3 or 1.4.

### BIBLIOGRAPHY

- BLUM, W. The determination of aluminium as oxide. *J. Am. Chem. Soc.*, **38**, 1282 (1916).  
 HILLEBRAND, W. F., AND LUNDELL, G. E. *Applied Inorganic Analysis*, p. 397, J. Wiley & Sons, New York (1929).  
 ROLLER, P. S. Colorimetric determination of aluminium with aurin tri-carboxylic acid. *J. Am. Chem. Soc.*, **55**, 2437-8 (1933).  
 KOLTHOFF, I. M., AND SANDELL, E. B. *Text-book of Quantitative Inorganic Analysis*, p. 305, Macmillan, New York (1936).

## 24. Iron

### A. GRAVIMETRIC

A slight excess of ammonium hydroxide precipitates iron in the fer-

ric state quantitatively. Aluminum, phosphate and silicate should be absent from the sample.

### 1. Reagents

1.1. Ammonium hydroxide, 1:1.

1.2. Ammonium chloride wash solution. Dissolve 1 g. of  $\text{NH}_4\text{Cl}$  in 100 ml. of distilled water.

### 2. Procedure

Heat a suitable size sample to boiling and add 1 ml. of concd.  $\text{HNO}_3$  to insure oxidation of the iron. Add 1:1  $\text{NH}_4\text{OH}$  slowly with constant stirring until a slight excess is present as shown by the odor of the solution. Allow the precipitate to settle. Wash the precipitate several times by decantation with hot distilled water. Filter through filter paper and wash the precipitate with hot  $\text{NH}_4\text{Cl}$  wash solution. Dry the precipitate and ignite the residue to constant weight at  $1000^\circ \text{C}$ .

ppm. Iron =  $[\text{mg. of } \text{Fe}_2\text{O}_3 \times 699.4] \div \text{ml. sample}$ .

### B. COLORIMETRIC-BIPYRIDINE METHOD

In dilute ferrous iron solutions, whose pH is between 3.5 and 8.5, 2,2' bipyridine (dipyridyl) produces a pink color suitable for colorimetric determination. Ferric iron present does not give any color. The method is sensitive to 0.05 ppm. of iron. The color is stable for six months.

### 1. Apparatus

One of the following pieces of equipment is required.

1.1. Pyrex test tubes, 20 ml. for visual color comparison.



1.2. Duboseq type colorimeter for visual color comparison.

1.3. Photoelectric filter photometer.

1.4. Photoelectric spectrophotometer.

## 2. Reagents

2.1. Iron-free distilled water for the preparation of reagents and dilution water.

2.2. Bipyridine solution. Dissolve 0.1 g. of 2,2' bipyridine in 100 ml. of 0.1 N HCl.

2.3. Sodium sulfite solution. Dissolve 10 g. of  $\text{Na}_2\text{SO}_3$  in 100 ml. of distilled water. Prepare freshly.

2.4. Hydrochloric acid, 5 N.

2.5. Mercuric chloride solution. Dissolve 5 g. of  $\text{HgCl}_2$  in 100 ml. of distilled water.

2.6. Iron standard solution. Dissolve 0.4318 g. of ferric ammonium sulfate  $(\text{NH}_4)_2\text{SO}_4 \cdot \text{Fe}_2(\text{SO}_4)_3 \cdot 24\text{H}_2\text{O}$  in a little distilled water, add 10 ml. of concd. HCl and dilute to 1 liter. Add 0.5 ml. of  $\text{HgCl}_2$  solution to 20 ml. of this solution and dilute to 1 liter. The final solution contains 0.001 mg. of iron per ml.

## 3. Procedure

3.1. Total iron. To 10 ml. of clear sample, add each of the following reagents with mixing: 2 ml. of dipyriddy solution, 1 ml. of  $\text{Na}_2\text{SO}_3$  solution, 0.1 ml. of 5 N HCl. Wait 5 minutes for full color development. Check the iron content of the reagents by carrying out the procedure on 10 ml. of iron-free water. Subtract the iron in the blank (if any), from the observed iron. Compare colorimetrically against standards prepared

simultaneously if using test tubes, or a Duboseq type colorimeter; or against a blank if using apparatus 1.3 or 1.4. A wavelength of 520 millimicrons is suitable for this photometric comparison.

3.2. Ferrous iron. Carry out the procedure as outlined under Total Iron but omit the sodium sulfite solution. The iron indicated is then ferrous iron.

## C. COLORIMETRIC—PHENANTHROLINE METHOD

Ortho-phenanthroline reacts with ferrous iron in dilute solution at a pH of 2.0–9.0 to give a pink to red color. The method is sensitive to 0.05 ppm. of iron. The color is stable for six months.

### 1. Apparatus

One of the following pieces of equipment is required.

1.1. Nessler tubes for visual color comparison.

1.2. Duboseq type colorimeter for visual color comparison.

1.3. Photoelectric filter photometer.

1.4. Photoelectric spectrophotometer.

### 2. Reagents

Iron-free distilled water for the preparation of reagents and dilution water.

2.1. Ortho-phenanthroline solution. Dissolve 0.12 g. of o-phenanthroline monohydrate in 100 ml. of distilled water. Solution is aided by heating to about 80° C. Two ml. of this solution is required for 0.1 mg. of iron.

2.2. Hydroxylamine solution. Dis-

solve 10 g. of hydroxylamine hydrochloride in 100 ml. of distilled water.

2.3. Hydrochloric acid, 1:9.

2.4. Standard iron solution. Dissolve 0.7022 g. of  $\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$  in 50 ml. of distilled water and 20 ml. of coned.  $\text{H}_2\text{SO}_4$  and dilute to one liter with distilled water. One ml. of the solution contains 0.1 mg. Fe.

### 3. Procedure

To a 50 ml. sample add 1 ml. of 1:9 HCl and boil to insure solution of all iron. Cool to room temperature and add 1 ml. of hydroxylamine solution and 2 ml. of ortho-phenanthroline solution. Add 0.5 ml. of coned.  $\text{NH}_4\text{OH}$ . The solution should be pink to congo red paper. Compare colorimetrically against standards prepared simultaneously if using Nessler tubes, or a Duboseq type colorimeter; or against a blank if using apparatus 1.3 or 1.4. Check the iron content of the reagents by carrying out the procedure on 50 ml. of iron-free water. Subtract the iron in the blank, if any is found, from the observed iron. A wavelength of 510 millimicrons is suitable for this photometric comparison.

### D. COLORIMETRIC—THIOCYANATE METHOD FOR TOTAL IRON

In colorimetric estimation of iron, the specific reagent (thiocyanate) shall be added to each of the iron standards at the same time that it is added to the prepared samples of water under examination. Comparison of color developed in both standards and samples shall be in matched Nessler tubes in the presence of equivalent concentrations of acid, immediately after the reagent is mixed

with the solutions, since the color is deepened by an excess of the reagent, is diminished by an excess of acid and fades quickly on standing.

### 1. Reagents

1.1. Standard ferric iron solution. Dissolve 0.7022 g. of crystallized ferrous ammonium sulfate  $[\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}]$  in 50 ml. of distilled water and 20 ml. of coned.  $\text{H}_2\text{SO}_4$ . Warm the solution and add  $\text{KMnO}_4$  soln. until the iron is completely oxidized as evidenced by the persistence of a faint pink color. Dilute to 1 liter; 1 ml. contains 0.1 mg. Fe.

1.2. Potassium (or ammonium) thiocyanate. Two g. dissolved to make 100 ml. of solution.

1.3. Hydrochloric acid, 1:3.

1.4. Potassium permanganate. Approximately 0.2 N. Dissolve 6.3 g. in distilled water and make up to 1 liter.

1.5. Hydrochloric acid. Concentrated, free from iron.

1.6. Nitric acid. Sp. gr. 1.42, free from iron.

1.7. Nitric acid. Sp. gr. 1.195; 382 ml. concentrated acid (sp. gr. 1.42) in 1 liter. Approximately 6 N.

### 2. Procedure

2.1. Normal procedure. Evaporate 100 ml. or less of the sample to dryness or use the residue on evaporation (Part I, Sec. 9, page 20). (With silt-bearing waters the quantity of iron is sometimes so great that it is necessary to use as little as 10 ml. of the sample. With such waters evaporation should be in the presence of 5 to 10 ml. of coned. HCl. If the sample contains much organic matter,

destroy this by ignition, taking care not to prolong ignition so as to render the iron too difficultly soluble.)

Heat the dish to drive off excess acid, if the residue was not ignited. Cool the dish and add 0.8 to 1 ml. of 3 N HCl. Warm on the water bath, taking care to avoid evaporation to dryness by adding small quantities of distilled water. Rinse the hot solution into a 50 ml. Nessler tube, filtering if necessary.

Add a drop or two of potassium permanganate solution; if the color of the permanganate does not persist for at least 5 minutes, add more, drop by drop. Cool and dilute to the mark with distilled water. With iron standards in readiness (3), add 5 ml. of thiocyanate solution to the sample and to the standards, mix and compare immediately. With permanent standards (4) comparison must immediately follow the addition of thiocyanate.

2.2 Waters low in organic matter. Boil 50 ml. of the sample with 5 ml. 6 N  $\text{HNO}_3$  for 5 minutes, add 3 drops of permanganate solution and cool. Add 5 ml. of thiocyanate and compare immediately with standards prepared from the standard iron solution with 6 N  $\text{HNO}_3$  instead of 3 N HCl.

2.3 Surface waters of slight organic content. Test as follows: Take a portion which contains at least 0.5 mg. of iron. Concentrate in a beaker with 2 to 3 ml. of concentrated  $\text{HNO}_3$ , with addition of permanganate if necessary to destroy organic matter, until a volume of about 50 ml. remains. To the hot liquid add ammonia in very slight excess and warm until the odor of ammonia is hardly

discernible. Filter and wash with water at 70° to 80° C. containing a little ammonia. Dissolve the precipitate on the filter and in the beaker in 4 ml. of concentrated HCl, collecting the filtrate in a 50 ml. Nessler tube. Cool, dilute to the mark, add 5 ml. thiocyanate and compare at once with standards.

### 3. Preparation of Iron Standards

Place in matched Nessler tubes measured volumes (0.5 to 4 ml.) of standard iron solution (1.1) covering the range of standards required for the determinations at hand; <sup>dilute</sup> ~~add~~ to about 40 ml., add 5 ml. of 3 N HCl and 1 or 2 drops of potassium permanganate solution to each tube; dilute each to the mark. To each of these tubes and to those containing the prepared samples to be tested, add 5 ml. thiocyanate solution, mix and compare immediately the color developed. These standards are not permanent.

### 4. Preparation of Permanent Standards

4.1. Potassium chloroplatinate,  $\text{K}_2\text{PtCl}_6$ . Dissolve 4 g. in distilled water, add 200 ml. of concentrated HCl and dilute to 1 liter with distilled water.

4.2. Cobaltous chloride,  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ . Dissolve 48 g. in distilled water, add 200 ml. of concentrated HCl and dilute to 1 liter with distilled water. It is essential that the cobaltous chloride contain the proper amount of water of crystallization.

Place in 50 ml. Nessler tubes the volumes of platinum and cobalt solutions specified in Table 9 and make



TABLE 9.—PREPARATION OF PERMANENT STANDARDS FOR THE DETERMINATION OF IRON \*

Milligrams of iron	No. of ml. platinum solution	No. of ml. cobalt solution
0.0	0.0	0.0
0.01	1.00	0.60
0.02	2.25	1.20
0.03	3.30	1.85
0.04	4.65	2.75
0.05	5.75	3.65
0.075	8.85	6.60
0.10	11.30	10.00
0.125	14.70	12.80
0.15	16.85	15.10

\* Revised in conformance with data in "The colorimetric determination of iron in water by the thiocyanate method." Hinman, J. J. and Mathews, E. A. Complete report is on file in the reference library of the National Research Council.

The color of these standards is not a precise spectroscopic match of the iron color. Where conditions permit, the use of temporary standards is preferable and the comparison of known and unknown is satisfactorily made in flat bottomed porcelain dishes.

up to the 50 ml. mark with distilled water. The iron equivalent of these standards is shown in the first column.

## BIBLIOGRAPHY

- KLUT. Mitt. Materialprüfungsamt, 12, 186 (1894).
- JACKSON, D. D. Permanent standards for use in the analysis of water. *Technology Quarterly (M. I. T.)*, 13, 320 (1900).
- KOLTHOFF, I. M., AND SANDELL, E. B. *Text-book of Quantitative Inorganic Analysis*, p. 299, Macmillan, New York (1936).
- ANON., *The B. D. H. Book of Reagents*, 5th Ed., p. 42, The British Drug Houses Ltd., London (1936).
- FORTUNE, W. B., AND MELLON, M. G. Determination of iron with o-phenanthroline. A spectrophotometric study. *Ind. Eng. Chem., Anal. Ed.*, 10, 60 (1938).
- WOODS, J. T., WITH MELLON, M. G. Thiocyanate method for iron. A spectrophotometric study. *Ind. Eng. Chem., Anal. Ed.*, 13, 551 (1941).
- MEHLIG, R. P., AND HULETT, R. H. Spectrophotometric determination of iron with o-phenanthroline and with nitro-o-phenanthroline. *Ind. Eng. Chem., Anal. Ed.*, 14, 869 (1942).

MOSS, M. L., WITH MELLON, M. G. Colorimetric determination of iron with 2,2'-bipyridyl and with 2,2', 2''-terpyridyl. *Ind. Eng. Chem., Anal. Ed.*, 14, 862 (1942).

## 25. Chromium

### A. COLORIMETRIC

Diphenyl carbazide produces a reddish-violet color with hexavalent chromium. Total chromium is obtained by the same reaction if chromic salts are oxidized to chromates with perchloric acid. Metal ions normally present in water do not interfere, except iron if present in amount greater than 1.0 ppm. For the colorimetric comparison, chromium should be present in concentrations between 0.005 and 0.4 ppm. A wavelength of 500 millimicrons is recommended for photometric estimation.

### 1. Apparatus

One of the following pieces of equipment is required.

- 1.1 Nessler tubes for visual color comparison.
- 1.2 Duboseq type colorimeter for visual color comparison.
- 1.3 Photoelectric filter photometer.
- 1.4 Photoelectric spectrophotometer.

### 2. Reagents

- 2.1. Perchloric acid, 60 per cent cp.
- 2.2. Ammonium hydroxide solution, 1:1.
- 2.3. Diphenyl carbazide solution. Dissolve 0.2 g. of diphenyl carbazide in 100 ml. of  $C_2H_5OH$ . Add 400 ml. of 1:9  $H_2SO_4$  and keep in a refrigerator.

2.4. Sulfuric acid, 1:1.

2.5. Potassium chromate standard solution. Dissolve 0.3740 g. of  $K_2CrO_4$  in 1 liter of distilled water. Dilute 10 ml. to 1 liter with distilled water. One ml. of dilute solution = 0.001 mg. Cr.

### 3. Procedure

3.1. Hexavalent chromium. Add 2.5 ml. of diphenyl carbazide reagent (2.3) to 50 ml. of sample in a Nessler tube, mix, and wait 5 minutes for full color development. Compare colorimetrically against standards prepared simultaneously if using apparatus 1.1 or 1.2; or against a blank if using apparatus 1.3 or 1.4.

3.2. Total chromium. Add 5 ml. of perchloric acid to a 50 ml. sample in an Erlenmeyer flask covered with a short stem funnel. Heat on a hot plate under a fume hood until white fumes of perchloric acid are evolved. Cool, add 25 ml. of distilled water and boil for 2 minutes. Cool and neutralize the solution with 1:1  $NH_4OH$ . Filter off any precipitate which may form. Just acidify the filtrate with 1:1  $H_2SO_4$ . Dilute to 50 ml. with distilled water. Add 2.5 ml. of diphenyl carbazide reagent and proceed as in 3.1.

### BIBLIOGRAPHY

- LICHTIN, J. J. Perchloric acid agent as oxidizing agent in the determination of chromium. *Ind. Eng. Chem., Anal. Ed.*, **2**, 126 (1930).
- GRAHAM, D. W. Chromium—a water and sewage problem. *J. Amer. W. W. Assn.*, **35**, 159 (1943).

## 26. Manganese

### A. COLORIMETRIC—PERIODATE METHOD

Strong oxidizing agents, such as sodium periodate and ammonium persulfate, oxidize manganous compounds to permanganates from which colorimetric estimation is made against standards prepared similarly. The concentration of manganese should be below 10 ppm. The periodate method is preferable if the manganese is present in very small amount.

#### 1. Reagents

1.1. Manganous sulfate standard solution. Dissolve 0.1438 g. of  $KMnO_4$  in distilled water to which has been added 2 to 3 ml. of 2 N  $H_2SO_4$ . Reduce by adding 0.4 g. of  $NaHSO_3$ . Boil off the excess  $SO_2$  and dilute to 1 liter with distilled water.

1.2. Sodium paraperiodate-sulfuric acid reagent. Prepare a  $H_2SO_4$  soln. by adding 120 ml. of concd.  $H_2SO_4$  to 1500 ml. of distilled water and diluting to 2 liters. Add 2.4 g. of  $Na_3H_2IO_6$ , heat to boiling and place, for 30 minutes, in a boiling water bath.

1.3. Manganese color standards. Add 1.2 ml. of concd.  $H_2SO_4$  and 30 ml. of sodium paraperiodate-sulfuric acid reagent to exactly 20 ml. of standard  $MnSO_4$  soln. After thoroughly mixing, add 0.3 g. of solid  $Na_3H_2IO_6$ , heat to boiling and place, for 30 minutes, in a boiling water bath. Cool and dilute to 1 liter with  $H_2SO_4$  reagent. This standard contains 0.001 g. of permanganate manganese per liter. Prepare a series of color standards in accordance with Table 10 and store in glass stoppered bottles which

have been cleaned with hot dichromate cleaning solution, washed and dried.

## 2. Procedure

Add 3 or 4 drops of concd.  $\text{H}_2\text{SO}_4$  to 100 ml. of the water sample and evaporate to dryness in an evaporating dish. Measure out 100 ml. of sodium paraperiodate-sulfuric acid reagent. With this, moisten the residue and transfer it to a 250 ml. Erlenmeyer flask. Then wash the dish thoroughly by repeated use of all the remaining acid reagent, pouring the washings into the flask. Add 0.3 g. of solid sodium paraperiodate, heat to boiling and place the flask in a boiling water bath for 30 minutes. Cool to room temperature and transfer the solution to a 100 ml. Nessler tube. Compare with standards and read directly in parts per million provided a 100 ml. portion of the sample was used for the test.

In all cases prepare a comparison tube containing no permanganate manganese, but 100 ml. of sodium

TABLE 10.—COLOR STANDARDS FOR DETERMINATION OF MANGANESE. USE 100 ML. COMPARISON TUBES

Permanganate manganese ml.	Reagent (1.2) ml.	ppm. of Mn when 100 ml. sample is used
0.0	100.0	0.0
2.5	97.5	0.025
5.0	95.0	0.05
7.5	92.5	0.075
10.0	90.0	0.1
20.0	80.0	0.2
30.0	70.0	0.3
40.0	60.0	0.4
50.0	50.0	0.5
60.0	40.0	0.6
70.0	30.0	0.7
80.0	20.0	0.8
90.0	10.0	0.9
100.0	0.0	1.0

paraperiodate-sulfuric acid solution as a blank, as this reagent has a color greater than that which might be interpreted as zero manganese.

## B. COLORIMETRIC—PERSULFATE METHOD

### 1. Reagents

1.1. Nitric acid, 1:1.

1.2. Silver nitrate solution. Dissolve 20 g. of  $\text{AgNO}_3$  in 1 liter of distilled water.

1.3. Manganous sulfate standard solution. Dissolve 0.2877 g. of  $\text{KMnO}_4$  in about 100 ml. of distilled water. Acidify the solution with  $\text{H}_2\text{SO}_4$  and heat to boiling. Add slowly, a sufficient quantity of dilute solution of oxalic acid to discharge the color. Cool and dilute to 1 liter with distilled water. One ml. of this solution contains 0.1 mg. of manganese.

1.4. Ammonium persulfate, crystals, free from chloride.

### 2. Procedure

Use an amount of the sample which contains not more than 0.2 mg. of manganese. Add 2 ml. of 1:1  $\text{HNO}_3$  and boil down to about 50 ml. Precipitate the chloride with the  $\text{AgNO}_3$  soln., adding at least 1 ml. in excess. Shake and heat to coagulate the precipitate, and filter. A sample which contains much chloride should be evaporated with a few drops of  $\text{H}_2\text{SO}_4$  until white fumes appear and then diluted before the  $\text{HNO}_3$  and  $\text{AgNO}_3$  are added as directed above.

If the sample is highly colored by organic matter it should be evaporated with  $\text{H}_2\text{SO}_4$ , and the residue ignited and dissolved in dil.  $\text{HNO}_3$ . Add about 0.5 g. of ammonium per-



sulfate crystals and warm the solution until the maximum permanganate color is developed. This usually takes about 10 minutes. At the same time prepare standards by diluting portions of 0.2, 0.4, 0.6 ml., etc., of the standard  $\text{MnSO}_4$  soln. to about 50 ml. and treating them exactly as the sample was treated. Transfer the sample and the standards to 50 ml. Nessler tubes, and compare the colors immediately.

## BIBLIOGRAPHY

RICHARDS, M. B. Colorimetric determination of manganese in biological material. *Analyt.*, 55, 554 (1930).

## 27. Zinc

After a preliminary separation from possible interfering substances in solution by hydrogen sulfide, zinc is determined nephelometrically in dilute solution by the addition of potassium ferrocyanide.

### A. NEPHELOMETRIC

#### 1. Reagents

- 1.1. Hydrochloric acid, 6 N.
- 1.2. Hydrochloric acid, 0.1 N.
- 1.3. Potassium hydroxide solution, 0.1341 N.
- 1.4. Thymol blue indicator solution, 0.04 per cent (see Part I, Sec. 11, B, page 30).
- 1.5. Brom phenol blue indicator solution, 0.04 per cent (see Part I, Sec. 11, B, page 30).
- 1.6. Sodium citrate, solid,  $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$ .
- 1.7. Copper sulfate, solution. Dissolve 3.939 g. of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in 1 liter of distilled water.
- 1.8. Potassium ferrocyanide solu-

tion. Dissolve 2 g. of  $\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$  in 100 ml. of distilled water.

1.9. Zinc sulfate standard solution. Dissolve 4.399 g. zinc sulfate ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ) in 1 liter of distilled water. Dilute 100 ml. to 1 liter with distilled water. One ml. of this solution contains 0.1 mg. of  $\text{Zn}^{++}$ .

## 2. Procedure

Acidify 1 liter of water with concd. HCl and evaporate to dryness in an evaporating dish. Extract the residue with HCl and filter. Dilute the filtrate to about 75 ml. and add 5 g. of pure sodium citrate, 2 ml. of  $\text{CuSO}_4$  soln. and a drop of thymol blue indicator. Add dilute KOH soln. until the solution becomes yellow, and then add a drop of brom phenol blue. If the solution is bluish at this point, add dilute acid until the yellow color is just restored. Saturate the cold solution with  $\text{H}_2\text{S}$  gas, filter out the zinc and copper sulfides, washing well to free from iron salts as much as possible. Dissolve the combined sulfides in  $\text{HNO}_3$  and HCl, dissolve the residue in HCl and repeat the precipitation as sulfide, omitting the addition of sodium citrate. In the absence of citrate buffer, the adjustment of acidity is much sharper and requires more care. Treat the reprecipitated sulfides as above, add 5 ml. of 6 N HCl to the residue, and 20 ml. of distilled water. Slowly saturate the cold solution with  $\text{H}_2\text{S}$  and filter. This separates the zinc from the copper, leaving the zinc in the filtrate.

Evaporate the filtrate to dryness and dissolve the residue in 4 to 5 drops of 6 N HCl and a little distilled

water. The solution of the residue at this point is occasionally refractory, and it is necessary to see that it is moistened throughout with acid and warmed slightly before the addition of water. Transfer to a 25 ml. volumetric flask.

To an aliquot portion (5 or 10 ml.) of this solution add 10 ml. of 0.1341 N KOH and carefully neutralize to phenolphthalein the excess KOH with 0.1 N HCl then adding exactly 1 ml. of acid in excess. Dilute with distilled water almost to 50 ml., add 1 ml. of potassium ferrocyanide solution in a Nessler tube and thoroughly mix at once. This solution is now 0.002 N with respect to acid, and 0.0268 molar with respect to potassium chloride.

The nephelometric standards in Nessler tubes should be prepared in exactly the same way in order to insure equivalent salt and acid concentrations. The most suitable range for comparison is that of standards containing 0.20 to 0.25 mg. per 50 ml. matched against solutions of the unknown of nearly the same opacity. Matched Nessler tubes of colorless glass should be used and it is an advantage to blacken the bottom and place a band about the meniscus. Especial attention should also be given to the quality of light, as it is difficult to obtain satisfactory readings in artificial light. The standards for comparison should vary from 0.05 to 0.50 mg. of zinc in steps of 0.05 mg.

#### BIBLIOGRAPHY

- FAIRHALL, L. T., AND RICHARDSON, J. R.  
The nephelometric analysis of zinc. *J. Am. Chem. Soc.*, 52, 938 (1930).

## 28. Calcium

### A. GRAVIMETRIC

Ammonium oxalate precipitates calcium quantitatively as calcium oxalate. The precipitate is ignited to and weighed as calcium oxide. The solution should be reasonably free from silica, phosphates, iron, aluminum, manganese, and should not contain more than 100 mg. of calcium.

#### 1. Reagents

- 1.1. Hydrochloric acid, 1:1.
- 1.2. Ammonium hydroxide, 1:1.
- 1.3. Ammonium hydroxide, 1:100.
- 1.4. Ammonium oxalate solution.  
Saturated solution of  $(\text{NH}_4)_2\text{C}_2\text{O}_4 \cdot \text{H}_2\text{O}$  at room temperature.
- 1.5. Methyl red indicator solution.

#### 2. Procedure

To 100 ml. of solution freed of silica, phosphates, iron, aluminum, and manganese, add a few drops of methyl red solution and acidify with 1:1 HCl until the color of the solution just changes to red. Heat to 70°–80° C. Add slowly hot  $(\text{NH}_4)_2\text{C}_2\text{O}_4$  solution in slight excess, then add 1:1  $\text{NH}_4\text{OH}$  until the color of the solution becomes yellow. Digest the precipitate for 1 hour at 80°–90° C. Filter off the precipitate of  $\text{CaC}_2\text{O}_4$  and wash with 1:100  $\text{NH}_4\text{OH}$ . Ignite the precipitate in a covered crucible (platinum preferably) at 1100°–1200° C. for 15 minute intervals to constant weight. Since CaO is highly hygroscopic, suitable precautions should be taken in cooling and weighing the crucible.

$$\text{ppm. Ca}^{++} = [\text{mg. CaO} \times 714.7] \div \text{ml. sample.}$$

## B. VOLUMETRIC

Ammonium oxalate precipitates calcium quantitatively as calcium oxalate which is then dissolved in sulfuric acid and titrated with standard potassium permanganate. The solution should be reasonably free from silica, phosphates, iron, aluminum, manganese and should not contain more than 100 mg. of calcium.

1. *Reagents*

- 1.1. Hydrochloric acid, 1:1.
- 1.2. Ammonium hydroxide, 1:1.
- 1.3. Ammonium hydroxide, 1:100.
- 1.4. Ammonium oxalate solution. Saturated solution of  $(\text{NH}_4)_2\text{C}_2\text{O}_4 \cdot \text{H}_2\text{O}$  at room temperature.
- 1.5. Methyl red indicator solution.
- 1.6. Potassium permanganate solution, 0.1 N  $\text{KMnO}_4$ .
- 1.7. Sulfuric acid, 1:8.

2. *Procedure*

Precipitate the Ca as  $\text{CaC}_2\text{O}_4$  as described in the gravimetric procedure. After washing the precipitate with 1:100  $\text{NH}_4\text{OH}$ , follow with a small amount of cold distilled water. Dissolve the washed precipitate with 25 ml. of 1:8  $\text{H}_2\text{SO}_4$  and dilute to 100 ml. with distilled water. Heat to  $80^\circ\text{C}$ . and titrate slowly while stirring with standard 0.1 N  $\text{KMnO}_4$  solution to a pink tinge.

ppm.  $\text{Ca}^{++} = [\text{ml. KMnO}_4 \text{ soln.} \times 2004] \div \text{ml. sample.}$

## BIBLIOGRAPHY

- HILLEBRAND, W. F., AND LUNDELL, G. E. *Applied Inorganic Analysis*, p. 497, J. Wiley & Sons, New York (1929).
- KOLTHOFF, I. M., AND SANDELL, E. B. *Textbook of Quantitative Inorganic Analysis*, pp. 337, 576, Macmillan, New York (1936).

AM. SOC. TEST. MTLs. Method for determination of the calcium ion and magnesium ion in industrial water. Method D 511-42, *A. S. T. M. Standards III*, 803 (1943).

## 29. Magnesium

## A. GRAVIMETRIC

Diammonium hydrogen phosphate precipitates magnesium quantitatively in ammoniacal solution as magnesium ammonium phosphate. The precipitate is ignited to, and weighed as magnesium pyrophosphate. The solution should be reasonably free from silica, phosphates, iron, aluminum, manganese, calcium, and preferably should not contain more than 100 mg. of magnesium as the oxide.

1. *Reagents*

- 1.1. Diammonium hydrogen phosphate solution. Dissolve 10 g. of  $(\text{NH}_4)_2\text{HPO}_4$  in 100 ml. of distilled water.
- 1.2. Ammonium hydroxide, 1:100.
- 1.3. Methyl red indicator solution.

2. *Procedure*

To the filtrate and washings secured after calcium removal (Part I, Sec. 28, A, page 59), add a few drops of methyl red solution, then carefully add coned. HCl until the methyl red indicator turns red. With constant stirring, add a slight excess of hot diammonium phosphate solution, followed by coned.  $\text{NH}_4\text{OH}$  until the methyl red indicator turns yellow. Heat the solution to boiling. With stirring, add 5 ml. of coned.  $\text{NH}_4\text{OH}$  in excess. Continue stirring for several minutes, then set aside for at least 4 hours digestion at room temperature. Filter and wash the pre-



precipitate with 1:100  $\text{NH}_4\text{OH}$ . Dry the filter paper and precipitate thoroughly. In a porcelain crucible burn off the filter paper slowly and carefully, at a low temperature. Then ignite at  $1100^\circ\text{C}$ . to constant weight.

ppm.  $\text{Mg}^{++} = [\text{mg. Mg}_2\text{P}_2\text{O}_7 \times 218.4] \div \text{ml. sample.}$

## B. COLORIMETRIC

In dilute magnesium solutions, in the presence of starch as a protective colloid, titan yellow forms a dispersed orange-red or red lake. The color of the lake has a minimum transmittance at a wavelength of approximately 525 millimicrons when compared with a blank. The solution should not contain more than approximately 10 ppm. Mg.

### 1. Apparatus

One of the following pieces of equipment is required.

- 1.1. Nessler tubes for visual color comparison.
- 1.2. Duboscq type colorimeter for visual color comparison.
- 1.3. Photoelectric filter photometer.
- 1.4. Photoelectric spectrophotometer.

### 2. Reagents

2.1. Magnesium-free distilled water for the preparation of reagents and dilution water.

2.2. Titan yellow solution. Dissolve 0.05 g. of titan yellow in 100 ml. of distilled water.

2.3. Calcium sulfate solution, saturated.

2.4. Starch solution. Dissolve 1 g. of soluble starch in 100 ml. of hot

distilled water. Make fresh every few days.

2.5. Sulfuric acid solution, 1:35.

2.6. Sodium hydroxide solution, 2 N.

2.7. Stock magnesium standard solution. Dissolve 2.5341 g. of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  in 500 ml. of distilled water. (1 ml. = 0.5 mg. of Mg.)

2.8. Dilute magnesium standard solution. Dilute 50 ml. of stock magnesium standard solution with distilled water to 500 ml. One ml. of the dilute solution contains 0.05 mg. of Mg.

### 3. Procedure

To a maximum of 25 ml. of clear solution, add each of the following reagents with mixing: 0.5 ml. of 1:35  $\text{H}_2\text{SO}_4$ , 4 ml. of starch solution, 10 ml. of  $\text{CaSO}_4$  soln., 1 ml. (for apparatus 1.1 or 1.2) or 5 ml. (for apparatus 1.3 or 1.4) of titan yellow solution and 5 ml. of 2 N NaOH. Dilute to 50 ml. Wait 5 minutes for full color development. Compare colorimetrically against standards prepared simultaneously if using apparatus 1.1 or 1.2; or against a blank if using apparatus 1.3 or 1.4.

## BIBLIOGRAPHY

- KOLTHOFF, I. M., AND SANDELL, E. B. *Textbook of Quantitative Inorganic Analysis*, p. 349, Macmillan, New York (1936).
- HILLEBRAND, W. F., AND LUNDELL, G. E. *Applied Inorganic Analysis*, p. 512, J. Wiley & Sons, New York (1929).
- AM. SOC. TEST. MTLs. Method for determination of the calcium and magnesium ions in industrial waters. Method D 511-42, *A. S. T. M. Standards, III*, 803 (1942).
- LUDWIG, E. E., AND JOHNSON, C. R. Spectrophotometric determination of magnesium by titan yellow. *Ind. Eng. Chem., Anal. Ed.*, 14, 895 (1942).

## 30. Sodium

## A. GRAVIMETRIC

Zinc uranyl acetate precipitates sodium as the triple acetate, sodium uranyl zinc acetate. The solubility of the precipitate in water is appreciable, hence the need for special care in the precipitation procedure. The reagent also precipitates phosphate which should therefore be absent or removed.

## 1. Reagents

1.1. Acetic acid solution. Dilute 150 ml. of glacial acetic acid with distilled water to 500 ml.

1.2. Uranyl zinc acetate solution. Mix equal volumes of solutions (a) and (b), allow to stand for 24 hours, then filter off the precipitate of sodium uranyl zinc acetate which forms from traces of sodium in the reagents. If no precipitate forms, add a small amount of sodium chloride to saturate the precipitant with the triple acetate. Store the solution in a Pyrex bottle.

(a) Dissolve 10 g. of  $\text{UO}_2(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 2\text{H}_2\text{O}$  in 20 ml. of acetic acid solution, add 50 ml. of water, and warm to dissolve.

(b) Dissolve 30 g. of  $\text{Zn}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 3\text{H}_2\text{O}$  in 10 ml. of acetic acid solution, add 50 ml. of water, and warm to dissolve.

1.3. Ethyl alcohol wash solution. Saturate 95 per cent ethyl alcohol with sodium uranyl zinc acetate prepared by adding NaCl to a portion of the uranyl zinc acetate solution.

1.4. Diethyl ether.

## 2. Procedure

Prepare by evaporation of the sample, or otherwise, a solution contain-

ing not more than 8 mg. of sodium and 25 mg. of potassium in 1 ml. of water in a 20 ml. Pyrex beaker. Add 10 ml. of uranyl zinc acetate solution, mix, and allow to stand covered for thirty to sixty minutes. Filter off the precipitate in a weighed medium porosity Pyrex fritted glass crucible, suck dry, and wash the precipitation beaker, crucible, and precipitate with five 2 ml. portions of the reagent; drain the crucible well each time. Then wash five times with 2 ml. portions of the alcohol wash solution, and finally with a few small portions of ether. Draw air through the crucible for a few minutes to volatilize the ether. Place the crucible in the balance case and weigh after 10 or 15 minutes. It is advisable to let the crucible stand on the balance pan for 10 minutes or so, and then weigh again to be sure that the weight is constant.

$$\text{ppm. Na}^+ = [\text{wt. of precipitate} \times 14.95] \div \text{ml. sample.}$$

## BIBLIOGRAPHY

KOLTHOFF, I. M., AND SANDELL, E. B. *Textbook of Quantitative Inorganic Analysis*, p. 395, Macmillan, New York (1936).

## 31. Potassium

## A. GRAVIMETRIC—PERCHLORATE METHOD

Potassium perchlorate is very slightly soluble in organic liquids such as a mixture of n-butyl alcohol and ethyl acetate whereas the corresponding sodium salt is soluble. Ammonium salts and sulfates should be absent from the solution.

### 1. Reagents

- 1.1. Perchloric acid, 60-70 per cent.
- 1.2. n-Butyl alcohol, cp.
- 1.3. Ethyl acetate, cp.

### 2. Preparation of Sample

Evaporate a suitable size sample to dryness. Acidify the residue with HCl, warm and filter off the silica. Add a hot solution of  $\text{BaCl}_2$  in slight excess to the hot filtrate and warm it, stirring at intervals for  $\frac{1}{2}$  hour, until the precipitate settles readily and leaves a clear supernatant liquid. Filter off the precipitate of barium sulfate.

Evaporate to dryness the filtrate from the barium sulfate and heat the residue barely to redness to remove ammonium salts. Take up in from 25 to 100 ml. of water, add a slight excess of saturated barium hydroxide solution and heat to boiling. Filter after  $\frac{1}{2}$  hour and wash the precipitate with hot water. Add to the filtrate  $\text{NH}_4\text{OH}$  and  $(\text{NH}_4)_2\text{CO}_3$  and heat on a steam bath till the calcium and barium carbonates settle, leaving clear liquid above the precipitate. Filter, evaporate the filtrate to dryness, and ignite at low red heat to expel ammonium salts. Take up in a few ml. of hot water, filter and wash, keeping the volume small. Repeat the addition of  $\text{NH}_4\text{OH}$  and  $(\text{NH}_4)_2\text{CO}_3$  and the succeeding operations till no precipitate is formed on addition of these reagents. Transfer the final filtrate to a small dish, preferably platinum, add a few drops of HCl and evaporate to dryness. Heat gently to drive off ammonium salts and finally heat barely to redness. Dissolve the

residue in as small a volume of water as possible.

### 3. Procedure

Add an excess of perchloric acid (1 ml. of 70 per cent perchloric acid is equal to 300 mg. of potassium) to the sample in a small beaker. Evaporate to dryness on the hot plate (not above  $350^\circ \text{C}$ ). If there is any acid on the side walls, "brush" the beaker with a flame to remove it. Cool, dissolve the residue in water (2 or 3 ml. will generally suffice), and again evaporate to dryness on the hot plate. To the cool residue add 10 to 20 ml. of a mixture of equal parts of anhydrous n-butyl alcohol and ethyl acetate, and digest near the boiling point for two to three minutes. Cool the solution to room temperature and decant the supernatant liquid through a weighed Gooch or porcelain filter crucible. Wash three times by decantation with 3 to 5 ml. portions of butyl alcohol-ethyl acetate. Dissolve the residue in a minimum volume of hot water, evaporate to dryness and extract as before, using 10 ml. of solvent. Then transfer the precipitate to the crucible, using a fine jet of the mixed solvent from a wash bottle. Wash the crucible and its contents approximately ten times with 1 ml. portions of butyl alcohol-ethyl acetate. Dry the beaker and brush any particles in it into the crucible. Place the crucible in an oven at  $110^\circ$  for a few minutes, and then heat at  $350^\circ$  for 15 minutes in a muffle. Cool and weigh as  $\text{KClO}_4$ .

ppm.  $\text{K}^+ = \text{mg. KClO}_4 \times 237.8 \div \text{ml. sample.}$



## B. COLORIMETRIC

Sodium cobaltinitrite precipitates potassium as dipotassium sodium cobaltinitrite. A sulfuric acid solution of the compound reduces potassium dichromate producing a color change suitable for indirect colorimetric determination of potassium. Ammonium ion should be absent.

### 1. Apparatus

One of the following pieces of equipment is required.

1.1. Nessler tubes for visual color comparison.

1.2. Duboseq type colorimeter for visual color comparison.

1.3. Photoelectric filter photometer.

1.4. Photoelectric spectrophotometer.

### 2. Reagents

2.1. Trisodium cobaltinitrite solution. Dissolve 10 g. of trisodium cobaltinitrite in 50 ml. of distilled water. Prepare fresh.

2.2. Potassium dichromate standard solution. Dissolve 4.9035 g. of dried  $K_2Cr_2O_7$  in 1 liter of distilled water. One ml. contains 1.321 mg.  $K^+$ .

2.3. Nitric acid, 1 N.

2.4. Nitric acid, 0.01 N.

### 3. Procedure

Add with mixing, 1 ml. of 1 N  $HNO_3$  and 5 ml. of trisodium cobaltinitrite solution to a 10 ml. sample containing from 1 to 7 mg. of potassium. Allow to stand for 2 hours. Centrifuge for 10 minutes. Decant and wash the precipitate with 15 ml. of 0.01 N  $HNO_3$ . Centrifuge, decant, add with mixing

10.0 ml. of  $K_2Cr_2O_7$  standard solution and 5 ml. of concd.  $H_2SO_4$ . Cool to room temperature. Make up the solution to 100 ml. with distilled water.

Check the potassium content of the reagents by carrying out the procedure on 10 ml. of potassium-free water. Subtract the potassium in the blank, if any is found, from the observed potassium. Compare colorimetrically against standards prepared simultaneously if using Nessler tubes, or a Duboseq type colorimeter; or against a blank if using apparatus 1.3 or 1.4. A wavelength of 425 millimicrons is suitable for this photometric comparison.

## BIBLIOGRAPHY

HICKS, W. B. A rapid modified chloroplatinate method for the estimation of potassium. *Ind. Eng. Chem.*, 5, 650 (1913).

SMITH, G. F., AND ROSS, J. F. The separation and determination of the alkali metals using perchloric acid. II, The precise estimation of the insoluble alkali metal perchlorates. *J. Am. Chem. Soc.*, 47, 774 (1925).

SMITH, G. FREDERICK, AND ROSS, J. F. The separation and determination of the alkali metals using perchloric acid. III. N butyl alcohol and ethyl acetate as mixed solvents in the separation and determination of potassium, sodium, and lithium. *J. Am. Chem. Soc.*, 47, 1020 (1925).

KOLTHOFF, I. M., AND SANDELL, E. B. *Textbook of Quantitative Inorganic Analysis*, p. 393, Macmillan, New York (1936).

WANDER, I. W. Photometric determination of potassium. *Ind. Eng. Chem., Anal. Ed.*, 14, 471 (1942).

## 32. Ammonia Nitrogen

Free ammonia is recovered quantitatively only when the distillation mixture is maintained at a pH of approximately 7.4. Natural waters exhibit varying pH values and buffering qualities. Consequently all waters require the addition of a phosphate buf-

fer solution to maintain a constant pH of the water during the distillation process. Direct Nesslerization may be resorted to with waters of high ammonia content. Results are reported in terms of nitrogen, save when knowledge of the ammonium content is essential, as in mineral analyses. The most reliable results are from determinations in fresh samples. (See Part I, Sec. 1, B, page 1.)

## A. DISTILLATION

### 1. Apparatus

1.1. Distillation shall be carried on in a glass flask with a vertical condenser, so arranged that the distillate shall drop directly from the block tin or aluminium tube into the receiving vessels.

1.2. Nessler tubes. The Nessler tubes (50 ml. capacity) shall conform to the specification in Part I, Sec. 2, C, page 4. Other optical comparison methods may be used.

### 2. Reagents

2.1. Ammonia-free water. Ammonia-free water may be made by re-distillation of distilled water that has been treated with bromine and allowed to stand overnight. For most work the ammonia may be removed from ordinary distilled water by shaking with Folin's ammonia permutit. Traces of magnesium, however, in some distilled water causes cloudy Nessler tubes. Cloudy tubes may be prevented by the use of Rochelle salt solution (2.3). Shake 1 gal. of tap water with 10 g. of Folin's ammonia permutit (the permutit may be used repeatedly) to absorb the ammonia;

decant and precipitate calcium and magnesium with 25 ml. of 25 per cent NaOH solution and 8 ml. of  $\text{CuSO}_4$  solution (10 per cent), shake, settle overnight and decant the clear liquor. This ammonia- and magnesium-free water does not give cloudy tubes on Nesslerization, and may be used in all steps of the Nesslerization process, but not being a distilled water, it cannot be used for making up reagents.

2.2. Nessler Reagent. Dissolve 61.75 g. of potassium iodide (KI) in 250 ml. of ammonia-free water, and add a cold solution of mercuric chloride ( $\text{HgCl}_2$ ) which has been saturated by boiling with an excess of the salt. Pour in the  $\text{HgCl}_2$  soln. cautiously, and add an amount just sufficient to make the color a permanent bright red.

With a little practice the exact depth of color may be easily duplicated. It will take a little more than 400 ml. of the  $\text{HgCl}_2$  soln. to reach this end point. Dissolve the red precipitate by adding exactly 0.75 g. of KI. Then add 150 g. of potassium hydroxide (KOH) dissolved in 250 ml. of water. Make up to 1 liter. Mix thoroughly and allow the precipitate to settle. Pour off the supernatant liquid. Mercuric chloride increases the sensitiveness of the reagent and potassium iodide decreases it.

This reagent should give the characteristic color with ammonia within 10 minutes after addition and should not produce a precipitate with small amounts of ammonia within two hours.

An alternate method of preparation is as follows: Dissolve 100 g. of  $\text{HgCl}_2$

and 70 g. of KI in a small quantity of ammonia-free water and add slowly, with stirring, to a cool solution of 160 g. of NaOH in 500 ml. of ammonia-free water. Dilute to 1 liter with ammonia-free water.

2.3. Potassium and sodium tartrate (Rochelle salt) solution. Dissolve 500 g. of cp. Rochelle salt ( $\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$ ) in 1 liter of water. Boil off 200 ml. or until free from ammonia. Cool and restore volume with ammonia-free distilled water.

2.4. Standard ammonium chloride solution. For the stock solution dissolve 3.818 g. of ammonium chloride in ammonia-free water and dilute to 1 liter. From this stock solution prepare the standard solution by diluting 10 ml. to 1 liter with ammonia-free water: 1 ml. contains 0.01 mg. of nitrogen, equivalent to 0.01288 mg. of  $\text{NH}_4$ .

2.5. 0.5 M phosphate buffer solution, pH 7.4. Dissolve 14.3 g. of monobasic potassium phosphate ( $\text{KH}_2\text{PO}_4$ ) and 90.15 g. of di-basic potassium phosphate ( $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$ ) in ammonia-free distilled water and make up to 1 liter of solution.

### 3. Procedure

Free the apparatus from ammonia by boiling distilled water in it until the distillate shows no trace of ammonia. Empty the distilling flask and measure into it 500 ml. of the sample, or a smaller measured volume diluted to 500 ml. with ammonia-free water. Add 10 ml. of phosphate-buffer solution and distill at a rate of not more than 10 ml. nor less than 6

ml. per minute, collecting the distillate in 50 ml. or larger, portions until ammonia-free.

Prepare a series of 16 Nessler tubes containing the following volumes of standard ammonium chloride solution, diluted to 50 ml. with ammonia-free water: namely, 0.0, 0.1, 0.3, 0.5, 0.7, 1.0, 1.4, 1.7, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 and 6.0 ml. The standards will contain 0.01 mg. of nitrogen for each 1 ml. of the standard solution.

Nesslerize the standards thus prepared and the portions of distillate by adding 1 ml. of Nessler reagent to each tube. (If the distillate has been collected in a flask, use an aliquot portion diluted to 50 ml. in a Nessler tube.) It is not necessary to stir the contents of the tubes after Nesslerizing. The temperature of both standards and prepared samples should be practically the same. Allow the tubes to stand at least 10 minutes after addition of the reagent; then compare the color produced in the portions of the distillate with that in the standards by looking through them at a white or mirrored surface so placed in front of a window that the light is reflected upward. It will be necessary to allow the tubes to stand for 30 minutes if the ammonium ion nitrogen is very low as it may be in condensed steam.

If the color resulting in a portion of distillate is greater than that of the darkest standard, mix the contents of the tube thoroughly, pour out half of the liquid and dilute the remainder to the original volume with ammonia-free water; make the color comparison and multiply the result by two. If the color is still too deep,



repeat the process of dilution until a reading can be made.

The sum of the corrected readings for the portions is equal to the number of mg. of ammonia nitrogen in the original volume of the sample distilled. If the distillate has been combined, and an aliquot portion Nesslerized, the observed result is to be multiplied by an appropriate factor.

## B. PREPARATION OF PERMANENT STANDARDS

### 1. Reagents

1.1. Potassium chloroplatinate  $K_2PtCl_6$ . Dissolve 2 g. of the salt in 300 to 400 ml. of distilled water, add 100 ml. of concd. HCl, and dilute to a liter with distilled water.

1.2. Cobaltous chloride  $CoCl_2 \cdot 6H_2O$ . Dissolve 12 g. of dry crystals in a small volume of distilled water, add 100 ml. of concd. HCl, and dilute to 1 liter.

### 2. Procedure

Measure into 50 ml. Nessler tubes the volumes of these solutions indicated in Table 11; dilute to the mark and mix thoroughly.

The values given in the table are approximate; actual equivalents of the standards thus prepared will differ with the quality of the Nessler reagent and the color sensitiveness of the analyst's eye. They should be compared with Nesslerized ammonia standards and the tints modified as necessary. Such comparisons should be made for each newly prepared Nessler solution and checked by each analyst.

These standards may be kept for several months if protected from dust.

TABLE 11.—PREPARATION OF PERMANENT STANDARDS FOR THE DETERMINATION OF AMMONIA NITROGEN

Value in ammonia nitrogen	Volume of platinum solution	Volume of cobalt solution
mg.	ml.	ml.
0.000	1.2	0.0
.001	1.8	.0
.002	2.8	.0
.004	4.7	.1
.007	5.9	.2
.010	7.7	.5
.014	9.9	1.1
.017	11.4	1.7
.020	12.7	2.2
.025	15.0	3.3
.030	17.3	4.5
.035	19.0	5.7
.040	19.7	7.1
.045	19.9	8.7
.050	20.0	10.4
.060	20.0	15.0
.070	20.0	22.0

They require no addition of reagent. Comparison with them is made at least 10 minutes after the distillate has been Nesslerized.

## C. DIRECT NESSLERIZATION

### 1. Reagents

1.1. Zinc sulfate. Dissolve 100 g. of zinc sulfate ( $ZnSO_4 \cdot 7H_2O$ ) in ammonia-free water and dilute to 1 liter.

1.2. Sodium or potassium hydroxide. Dissolve 50 g. in a little ammonia-free water, and dilute to 100 ml.

### 2. Procedure

Add 1 ml. of  $ZnSO_4$  soln. to 100 ml. of the sample, diluted if necessary with an equal volume of ammonia-free water, in a glass-stoppered graduated cylinder; mix thoroughly, add 0.4 to 0.5 ml. of alkali hydroxide solution, to obtain a pH of 10.5 and again mix

thoroughly. Allow the treated sample to stand for a few minutes, when a heavy precipitate should fall, leaving the supernatant liquid clear and colorless; or clarify by centrifuging or filtering through filter paper, discarding the first 25 ml. of filtrate. Nesslerize an aliquot portion, and compare with standards as directed in A, 3, page 66.

#### BIBLIOGRAPHY

- WESTON, R. S. Apparatus for the determination of ammonia in water, by the Wanklyn method, and total nitrogen by the Kjeldahl method. *J. Am. Chem. Soc.*, **22**, 469 (1900).
- JACKSON, D. D. Permanent standards for use in the analysis of water. *Technology Quarterly (M. I. T.)*, **13**, 314 (1900).
- PHELPS, E. B. A critical study of the methods in current use for the determination of free and albuminoid ammonia in sewage. *Pub. Health Papers and Repts., Am. Pub. Health Assn.*, **29**, 354 (1903); *J. Infect. Dis.*, **1**, 327 (1904).
- NICHOLS, M. S., AND FOOTE, M. E. Distillation of free ammonia nitrogen from buffered solutions. *Ind. Eng. Chem., Anal. Ed.*, **3**, 311 (1931).
- FOOTE, M. E., AND NICHOLS, M. S. The effect of hydrogen-ion concentration on the distillation of free ammonia nitrogen from sewages and trade wastes. *Sew. Wks. J.*, **4**, 37 (1932).

### 33. Albuminoid Nitrogen

Albuminoid nitrogen is the nitrogen equivalent of ammonia formed or liberated from nitrogenous matter by the action of alkaline permanganate in water after expulsion of ammonia nitrogen by distillation. It bears a variable ratio to organic nitrogen in highly polluted waters, and in waters of apparently slight pollution the values obtained by this method are related to color and colloidal matter.

#### 1. Reagent

Alkaline potassium permanganate. Boil 1.2 liter of distilled water in a 2.5 liter porcelain dish 10 minutes to drive off ammonia. Add 16 g. of potassium permanganate and stir until solution is effected. Add 800 ml. of clarified 9 N solution of potassium or sodium hydroxide, and ammonia-free water to make up to 2.5 liters. Concentrate to 2 liters. Determine the ammonia in 50 ml. of the reagent and use the result as a basis for correction in subsequent determinations.

#### 2. Procedure

Add 50 ml. or more of alkaline permanganate solution to the contents of the distilling flask after removal of ammonia from the sample (Part I, Sec. 32, A, 3, page 66), and continue distillation until four (preferably five) 50 ml. portions of distillate have been collected. Nesslerize and compare with standards. If the distillate has been collected in a 200 or 250 ml. flask, Nesslerize an aliquot portion diluted to 50 ml.

### 34. Organic Nitrogen

The Kjeldahl method of determining organic nitrogen, using copper sulfate as a catalyst and potassium or sodium sulfate to raise the boiling point of the sulfuric acid is recommended as the standard procedure to effect the complete destruction of organic matter with conversion of all nitrogen to ammonia.

#### 1. Reagents

1.1. Concentrated sulfuric acid, low in nitrogen.

1.2. Copper sulfate solution. Dissolve 100 g. of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in ammonia-free water and dilute to 1 liter.

1.3. Sodium hydroxide solution. Dissolve 500 g. of  $\text{NaOH}$  in ammonia-free water and dilute to 1 liter.

## 2. Procedure

Boil 500 ml. of the sample in a round-bottomed flask to remove ammonia (Part I, Sec. 32, A, 3, page 66); this usually causes the loss of 200 ml. of the sample, which may be collected for the determination of ammonia nitrogen. Add 10 ml. of nitrogen-free concd.  $\text{H}_2\text{SO}_4$  and 1 ml. of  $\text{CuSO}_4$  soln. Mix by shaking. Digest under a hood until copious fumes of sulfuric acid are given off and the liquid becomes colorless or a pale straw color. If necessary the digestion temperature may be raised by addition of 5 g. anhydrous potassium or sodium sulfate. Cool. Dilute to 300 ml. with ammonia-free water. Make alkaline with strong ammonia-free  $\text{NaOH}$ . Distill the ammonia and complete its estimation as directed in Part I, Sec. 32, page 64.

## BIBLIOGRAPHY

- PALMER, A. W. Report of the University of Illinois [in *Report of Streams Examinations, Sanitary Dist. Chicago*], 60 (1903).
- PHELPS, E. B. The determination of organic nitrogen in sewage by the Kjeldahl process. *J. Infect. Dis., Supp. 1*, 255 (1905).
- REPORT OF REFEREE ON ANALYSIS OF FERTILIZER. *J. Assn. Off. Agr. Chem.*, 4, 540 (1921).

## 35. Nitrate Nitrogen

Nitrate in waters is commonly reported in terms of the nitrogen equivalent, but in mineral analyses

results may be reported in terms of the acid radicle  $\text{NO}_3$ . Determination of nitrate content shall be made on fresh samples or on samples which have been sterilized by appropriate reagents, because conversion of nitrite into nitrate by bacterial action proceeds uninterruptedly. (See Part I, Sec. 1, B, page 1.)

## A. PHENOLDISULFONIC ACID METHOD

### 1. Reagents

1.1. Phenoldisulfonic acid. Dissolve 25 g. of pure white phenol in 150 ml. of pure concd.  $\text{H}_2\text{SO}_4$ . Add 75 ml. of fuming sulfuric acid (15 per cent free  $\text{SO}_3$ ), stir well, and heat for 2 hours at about  $100^\circ \text{C}$ .

1.2. Potassium hydroxide solution. Prepare an approximately 12 N solution, 10 ml. of which will neutralize about 4 ml. of the phenoldisulfonic acid.

1.3. Standard nitrate solution. Dissolve 0.7216 g. of pure recrystallized potassium nitrate in 1 liter of distilled water. Evaporate 50 ml. of this solution to dryness on the water bath. Moisten the residue quickly and thoroughly with 2 ml. of phenoldisulfonic acid, and rub with a glass rod to insure intimate contact. Dilute to 500 ml.; this is the standard solution, 1 ml. of which contains 0.01 mg. of nitrate nitrogen, or 0.04427 mg. of  $\text{NO}_3$ .

1.4. Standard silver sulfate solution. Dissolve 4.397 g. of silver sulfate free from nitrate in 1 liter of water; 1 ml. of this solution is equivalent to 1 mg. of chloride radicle.

1.5. Aluminum hydroxide. Electrolyze ammonia-free water, using aluminum electrodes. Wash the pre-



precipitate until it is free from chloride, ammonia, and nitrite. Or dissolve 125 g. of potassium or ammonium alum in 1 liter of distilled water. Precipitate the aluminum by adding cautiously ammonium hydroxide. Wash the precipitate in a large jar by successive additions and decantations of distilled water until free from chloride, nitrite, and ammonia.

## 2. Procedure

Measure into an evaporating dish 100 ml. of the sample. A smaller volume may be used unless the amount of nitrate is low, and in any case the volume of sample should be such that the nitrate nitrogen does not exceed 1 mg. The alkalinity, chloride, nitrite content, and color of the sample must be determined.

If nitrite nitrogen is present in excess of 1 ppm., it should be oxidized by heating the sample a few minutes with a few drops of hydrogen peroxide, free from nitrate, repeatedly added, or dilute  $\text{KMnO}_4$  may be added until a faint pink coloration persists; the nitrogen equivalent of the nitrite thus oxidized is then subtracted from the final nitrogen reading.

If the sample has a color over 10, decolorize it by adding 3 ml. of aluminum hydroxide suspension, stir very thoroughly, allow to stand for a few minutes, filter and wash with distilled water. Add sufficient 0.02 N  $\text{H}_2\text{SO}_4$  to neutralize the methyl orange alkalinity.

Then, if the chloride is above 30 ppm., or 3 mg. in the portion of the sample being examined, add to the cold solution sufficient standard silver sulfate solution to precipitate all but

about 0.1 mg. of chloride. Do not use an excess of silver sulfate since most waters, if heated with silver sulfate solution, suffer an appreciable loss of nitrate nitrogen.

Filter. Evaporate the filtrate to dryness. If the residue becomes packed, or appears vitreous because of the presence of much iron, heat the dish on the water bath for a few minutes. Add 2 ml. of disulfonic acid solution, rubbing with a glass rod to insure intimate contact.

Dilute the mixture with distilled water and add slowly a strong KOH soln. until the maximum color is developed. If nitrate is present, a yellow color will appear. Transfer the solution to a Nessler tube, filtering if necessary.

Compare the color with that of standards, made by adding 2 ml. of strong KOH soln. to various volumes of standard nitrate solution and diluting them to 50 ml. in Nessler tubes; the following volumes of standard nitrate solution are suggested: 0.1, 0.3, 0.5, 0.7, 1.0, 3.5, 10, 20, 30, 40, 50 ml., yielding standards containing 0.001 to 0.5 mg. nitrogen. These standards may be kept several weeks without deterioration. Standards prepared from tri-potassium nitrophenol disulfonate will remain permanent for several years if stored in the dark.

The nitrate plus the nitrite present expressed as ppm. is:

$$\text{ppm. N} = (1000 \times \text{mg. of N in the matched std.}) \div \text{ml. of sample used.}$$

## B. REDUCTION METHOD

### 1. Reagents

1.1. Sodium or potassium hydroxide. Dissolve 250 g. of the hydroxide

low in nitrogen in 1.25 liter of distilled water. Add several strips of aluminum foil and allow the evolution of hydrogen to continue overnight. Concentrate the solution to 1 liter by boiling.

1.2. Aluminum foil. Use strips of pure aluminum sheet about 10 cm. long, 6 mm. wide, and 0.33 mm. thick, weighing about 0.5 g.

## 2. Procedure

To 100 ml. or less of the sample in a 300 ml. casserole add 2 ml. of the hydroxide solution and concentrate by boiling to about 20 ml. Pour the contents of the casserole into a test tube about 16 cm. long and 3 cm. in diameter, of approximately 100 ml. capacity. Rinse the casserole several times with nitrogen-free water and add the rinse water to the liquid already in the tube, thus making the contents of the tube approximately 75 ml.

Add a strip of aluminum foil; close the tube by means of a rubber stopper through which passes a bent glass tube about 5 mm. in diameter. Put the shorter arm of the tube through flush with the lower side of the rubber stopper and let the longer arm extend below the surface of distilled water in another test tube. This apparatus serves as a trap through which evolved hydrogen escapes freely. The small amount of ammonia escaping into the trap may be neglected.

Allow the action to proceed for a minimum period of 4 hours, or overnight. Pour the contents of the tube into a distilling flask, dilute with 250 ml. of ammonia-free water, distill and collect the distillate in a 200 ml. flask,

and Nesslerize an aliquot part. If the supernatant liquid in the reduction tube is clear and colorless, the solution may be diluted to a definite volume and an aliquot part Nesslerized without distillation. Erroneous results may be obtained on highly polluted waters and sewage filter effluents due to the hydrolysis of urea or other amino compounds.

## BIBLIOGRAPHY

- HAZEN, A., AND CLARK, H. W. On the determination of nitrates in water. *Chem. News*, 64, 162 (1891):
- JACKSON, D. D. Permanent standards for use in the analysis of water. *Technology Quarterly (M. I. T.)*, 13, 314 (1900).
- KENDALL, L. M., AND RICHARDS, E. H. Permanent standards in water analysis. *Technology Quarterly (M. I. T.)*, 17, 277 (1904).
- TATLOCK, R. R., AND THOMPSON, R. T. The analysis of waters and their changes in composition when employed in steam raising. *J. Soc. Chem. Ind.*, 23, 428 (1904).
- CHAMOT, E. M., AND PRATT, D. S. A study on the phenolsulfonic acid method for the determination of nitrates in water. *J. Am. Chem. Soc.*, 31, 922 (1909); 32, 630 (1910); AND REDFIELD, H. W., 33, 366; 381 (1911).
- BARTOW, E., AND ROGERS, J. S. Determination of nitrates by reduction with aluminum. *Am. J. Pub. Hygiene*, new ser., 5, 536 (1909); also *Univ. Ill. Bull.*, 7 (Water Survey Series, 7), 14 (1909).

## 36. Nitrite Nitrogen

Determination of the nitrite content shall be made on fresh samples because conversion of nitrite into nitrate and into ammonia by bacterial action proceeds uninterruptedly. (See Part I, Sec. 1, B, page 1.) Results are usually reported in terms of the nitrogen equivalent.

## 1. Reagents

1.1. Sulfanilic acid solution. Dissolve 8 g. of cp. sulfanilic acid in 1 liter of 5 N acetic acid (sp. gr. 1.041). This is practically a saturated solution.

1.2.  $\alpha$ -Naphthylamine acetate solution. Dissolve 5 g. of solid  $\alpha$ -naphthylamine in 1 liter of 5 N acetic acid. Filter the solution periodically through washed absorbent cotton to remove decomposition products.

1.3. Sodium nitrite stock solution. Dissolve 1.1 g. of silver nitrite in nitrite-free water; precipitate the silver with sodium chloride solution and dilute to 1 liter. Settle for 48 hours and pipette off clear supernatant as required.

1.4. Standard sodium nitrite solution. Dilute 100 ml. of solution (1.3) to 1 liter, then dilute 50 ml. of this solution to 1 liter with sterilized nitrite-free water, add 1 ml. of chloroform, and preserve in a sterilized bottle. One ml. = 0.0005 mg. N = 0.001642 mg.  $\text{NO}_2$ .

An alternate method of preparation is to dissolve 0.246 g. of pure  $\text{NaNO}_2$  in 1 liter of nitrite-free water. Dilute 10 ml. of this solution to 1 liter with nitrite-free water. One ml. of the dilute standard solution contains 0.0005 mg. of nitrogen.

1.5. Aluminum hydroxide. (See Part I, Sec. 35, A, 1.5, page 69.)

1.6. Fuchsin solution. 0.1 g. of basic fuchsin per liter.

## 2. Procedure

Place in a standard Nessler tube 50 ml. of the sample, decolorized if necessary with nitrite-free aluminum hydroxide or a smaller amount diluted

to 50 ml. At the same time prepare in Nessler tubes a set of standards, by diluting to 50 ml. with nitrite-free water, various amounts of standard nitrite. The following volumes of standard solution are suggested: 0.0, 0.1, 0.2, 0.4, 0.7, 1.0, 1.4, 1.7, 2.0, and 2.5 ml. (containing 0.0005 mg. N per ml.).

Add 1 ml. of sulfanilic acid solution. Allow to stand 5 minutes and then add 1 ml. of the  $\alpha$ -naphthylamine acetate solution to the sample and to each standard. Mix thoroughly and allow to stand 10 minutes; then compare the sample with the standards. Do not allow the sample to stand more than 30 minutes before making the comparison. If the color of the sample is deeper than that of the highest standard repeat the test on a diluted sample.

The determination of nitrites according to the above procedure does not give satisfactory results when large quantities of chlorine have been added to water in process of treatment. Under these special conditions it is advisable to add first the  $\alpha$ -naphthylamine acetate and then the sulfanilic acid.

Permanent standards may be prepared by matching the nitrite standards with dilutions of the fuchsin solution. Fuchsin standards have been found to be sufficiently accurate for waters high in nitrite and for sewage. The standards should be checked once a month and kept out of the bright sunlight.

## BIBLIOGRAPHY

LOS VAY, M. L. Nitrous acid in the saliva and in exhaled air. *Bull. de la Soc. Chem.*, ser. 3, 2, 388 (1889).



WESTON, R. S. Notes on the determination of nitrogen as nitrites, in waters. *J. Am. Chem. Soc.*, 27, 281 (1905).

## 37. Chloride

### A. VOLHARD METHOD

Silver nitrate precipitates chlorides in acid solution as silver chloride. In the presence of nitrobenzene and ferric alum indicator the excess silver nitrate can be titrated with thiocyanate without removing the precipitated silver chloride. Phosphates and sulfites do not interfere.

#### 1. Reagents

1.1. Silver nitrate solution, 0.01711 N. Dissolve 2.9068 g. of dried  $\text{AgNO}_3$  in 1 liter of chloride-free distilled water. One ml. of this solution is equivalent to 1 mg. of NaCl. If necessary, standardize this solution against standard sodium chloride solution.

1.2. Sodium chloride standard solution. Dissolve 5.0000 g. of fused NaCl in 500 ml. of chloride-free distilled water. Dilute 10 ml. of this solution with chloride-free distilled water to a final volume of 100 ml. One ml. of this solution is equivalent to 1 mg. of NaCl.

1.3. Nitric acid, 1:1.

1.4. Potassium thiocyanate solution. Dissolve 2 g. of KCNS in 1 liter of chloride-free distilled water. Standardize this solution against the 0.01711 N  $\text{AgNO}_3$  soln.

1.5. Ferric alum solution, saturated.

#### 2. Procedure

To a clear 100 ml. sample add 5 ml. of 1:1  $\text{HNO}_3$ . Add, with stirring, 0.01711 N  $\text{AgNO}_3$  soln. until an excess of approximately 2 ml. is present, ac-

curately measuring the amount added. Add 3 ml. of nitrobenzene and 1 ml. of ferric alum indicator solution. Shake vigorously to coagulate the precipitate. Titrate the excess silver nitrate by adding standardized potassium thiocyanate until a permanent reddish brown color appears that does not fade after five minutes.

ppm.  $\text{Cl}^-$  as  $\text{Cl}^- = [(\text{ml. AgNO}_3 \text{ for sample} - \text{ml. AgNO}_3 \text{ for thiocyanate}) \times 606.6] \div \text{ml. sample.}$

ppm.  $\text{Cl}^-$  as NaCl =  $[(\text{ml. AgNO}_3 \text{ for sample} - \text{ml. AgNO}_3 \text{ for thiocyanate}) \times 1000] \div \text{ml. sample.}$

### B. MOHR METHOD

In a solution, whose pH is adjusted between the limits indicated, by methyl orange and phenolphthalein indicators, and to which potassium chromate is added as an indicator, silver nitrate combines first with all the chloride present, after which the red color of silver chromate appears. The concentration of chloride ion should be between 5 and 200 ppm. in the portion titrated. Sulfite, phosphate, and cyanide should be absent from the solution.

#### 1. Reagents

1.1. Silver nitrate solution, 0.01711 N. Same as 1.1 under A.

1.2. Sodium chloride standard solution. Same as 1.2 under A.

1.3. Potassium chromate indicator solution. Dissolve 50 g. of  $\text{K}_2\text{CrO}_4$  in a little distilled water. Add silver nitrate to produce a slight red precipitate. After standing, at least overnight, filter and dilute to 1 liter with distilled water.

1.4. Aluminum hydroxide suspen-

sion. Dissolve 125 g. of potassium or ammonium alum in 1 liter of distilled water. Precipitate the aluminum by adding ammonium hydroxide slowly and with stirring. Wash the precipitate by successive decantation of distilled water until free from chloride, nitrite, and ammonia.

1.5. Hydrogen peroxide, 30 per cent.

1.6. Phenolphthalein indicator solution.

## 2. Procedure

If necessary, decolorize the sample with the aluminum hydroxide suspension. Filter. If sulfites are present, add with stirring 1 ml. of  $\text{H}_2\text{O}_2$  soln. Adjust the sample with either dilute acid or dilute base so that it is just colorless to phenolphthalein.

To a suitable size sample, containing preferably between 10 and 100 ppm. chloride as  $\text{NaCl}$ , add 1 ml. of  $\text{K}_2\text{CrO}_4$  indicator soln. in a white porcelain evaporating dish or an Erlenmeyer flask over a white surface. Titrate with  $\text{AgNO}_3$  soln. until a color change from yellow to red is perceptible.

Determine the indicator blank by titrating with  $\text{AgNO}_3$  the same volume of chloride-free distilled water using 1 ml. of indicator solution; or use the same volume of chloride-free distilled water containing about 200 mg. of chloride-free calcium carbonate.

ppm.  $\text{Cl}^-$  as  $\text{Cl}^- = [(\text{ml. AgNO}_3 \text{ for sample} - \text{ml. AgNO}_3 \text{ for indicator blank}) \times 606.6] \div \text{ml. sample.}$

ppm.  $\text{Cl}^-$  as  $\text{NaCl} = [(\text{ml. AgNO}_3 \text{ for sample} - \text{ml. AgNO}_3 \text{ for indicator blank}) \times 1000] \div \text{ml. sample.}$

## BIBLIOGRAPHY

- HAZEN, ALLEN. On the determination of chlorine in water. *Am. Chem. J.*, 11, 409 (1889).  
 SCOTT, W. W. *Standard Methods of Chemical Analysis*, 4th Ed., pp. 131 and 154, Van Nostrand, New York (1925).  
 RIFFENBURG, H. B. Colorimetric determination of small quantities of chlorides in waters. *Ind. Eng. Chem., Anal. Ed.*, 7, 14 (1935).  
 CALDWELL, J. R., AND MOYER, H. V. Chloride determination. *Ind. Eng. Chem. Anal., Ed.* 7, 38 (1935).

## 38. Iodide

The iodide content of waters may be estimated by the modified McClendon method. This method depends on the liberation of iodine from iodide by nitrosyl-sulfuric acid and extraction with carbon tetrachloride.

### 1. Reagents

1.1. Sodium hydroxide solution, approximately 0.1 N.

1.2. Phosphoric acid approximately 0.1 N.

1.3. Arsenous acid, approximately 0.1 N ( $\text{As}_2\text{O}_3$ ).

1.4. Carbon tetrachloride. Color technical  $\text{CCl}_4$  with chlorine or bromine, allow it to stand for a week in sunlight, wash out the chlorine or bromine with  $\text{NaOH}$  soln., then with water; separate by first syphoning off the water, then filter the remaining liquid through a layer of plaster of paris. Distill, rejecting the cloudy first portion of distillate.

1.5. Chloroform, cp. If chloroform is used, stabilize in a manner equivalent to that used for carbon tetrachloride.

1.6. Nitrosyl-sulfuric acid solution. In a side arm distilling flask heated by a water bath, place a starch

paste made by mixing 50 g. of starch and an equal amount of water. Allow  $\text{HNO}_3$  (sp. gr. 1.35), to run in from a dropping funnel in the stopper onto the hot starch just fast enough to keep a steady stream of oxides of nitrogen coming over through a delivery tube connected to the side arm of the flask and dipping into 30 ml. of concd.  $\text{H}_2\text{SO}_4$ . The product is a nearly saturated solution of nitrosyl-sulfuric acid in sulfuric acid. In a closed bottle it keeps indefinitely.

1.7. Potassium iodide solution. Dissolve 13.5 mg. of KI in 1 liter of distilled water. (1 ml. = 0.01 mg. I.)

## 2. Procedure

Make alkaline, with sodium carbonate, 50 liters of the sample water (100 liters if the iodine content is low). Evaporate in a large evaporating dish into which more of the sample may be syphoned from a large tank or carboy as it boils away. The crust of calcium salts or residue left in the tank or carboy is discarded.

When the evaporation has proceeded until approximately 1 liter remains, filter out the solids, and evaporate further to 50 ml., filter into a nickel or platinum dish, washing the residue with a few ml. of hot water, and evaporate to dryness over a water bath or in an oven at  $110^\circ \text{C}$ .

Pulverize the residue and transfer the dry powder to a nickel boat. Insert the boat into the center of the Pyrex combustion tube which has a drawn out portion one-quarter inch in diameter on one end which is bent down and passes through one hole of a two-hole stopper into 30 ml. of 0.1 N NaOH soln. in a Pyrex test tube.

Through the other hole in the stopper place a piece of right angled glass tubing which does not reach down to the liquid in the test tube, so that when suction from a small filter pump is applied to this tube the air being drawn through the combustion tube will bubble up through the sodium hydroxide solution. With the suction pulling air through the tube at the rate of 3 or 4 bubbles per second, heat the tube and boat carefully, avoiding fusion of the ash.

The powder will turn black, then white, showing that the combustion is complete. If the alkaline solution becomes very discolored, it must be evaporated and burned again, but the ash will not have to be treated again.

Transfer the ash from the boat to a 100 ml. beaker and rinse out the tube with the alkaline solution, pouring rinsings into the beaker. Boil the solution, stirring the residue until it is dissolved or thoroughly leached out. Evaporate to about 20 ml. and filter. Neutralize the filtrate with phosphoric acid, using phenol red paper for the indicator.

Add a drop of arsenous acid and transfer to a 30 ml. separatory funnel. Add phosphoric acid to a yellow color with brom phenol blue test paper; then add 1 ml. of purified carbon tetrachloride, or chloroform, and a drop of nitrosyl-sulfuric acid, shake 100 times and allow to settle. Drain off the carbon tetrachloride into a 5 ml. test tube. Repeat the extraction adding the second ml. of carbon tetrachloride, or chloroform, to the first in the small tube. Compare the carbon tetrachloride solution with standards.



The standards are made by diluting 0.5, 1.0, 5.0, 10.0 and 15.0 ml. of the standard KI soln. to 20 ml. Each solution is placed in a small separatory funnel and a drop of 0.1 N arsenous acid added. Two extractions with carbon tetrachloride and nitrosyl-sulfuric acid are made and the carbon tetrachloride, or chloroform, solutions are drained into tubes similar to the one containing the iodine extracted from the water sample.

The prepared standards represent 5, 10, 100, 150 and 200 thousandths of a mg. of iodine in the 50 liter sample. Therefore, divide the result by the number of liters evaporated and report as parts per billion of iodine.

#### BIBLIOGRAPHY

HINMAN, J. J. Report of the Committee on Standard Methods of Water Analysis. *J. Amer. W. W. Assn.*, 19, 566 (1928).

### 39. Fluoride

#### A. WITHOUT DISTILLATION

This procedure is essentially that of Sanchis as modified by Scott. It depends on the formation of an unionized zirconium fluoride salt, thus decreasing the color of a zirconium alizarine lake. It has been found reliable for potable waters of ordinary composition. Up to the following limits it is not interfered with by: Chloride ion ( $\text{Cl}^-$ )—500 ppm., sulfate ion ( $\text{SO}_4^{--}$ )—200 ppm., alkalinity (expressed as  $\text{CaCO}_3$ )—200 ppm., acidity (expressed as  $\text{CaCO}_3$ )—200 ppm., iron ( $\text{Fe}$ )—2 ppm., aluminum ( $\text{Al}$ )—0.5 ppm., phosphate ion ( $\text{PO}_4^{=}$ )—1 ppm., color—25, and turbidity—25.

#### 1. Reagents

1.1. Acid zirconium alizarin reagent. Dissolve 0.3 g. zirconium oxychloride ( $\text{ZrOCl}_2 \cdot 8\text{H}_2\text{O}$ ) in 50 ml. distilled water contained in a 1 liter glass-stoppered flask. Dissolve 0.07 g. alizarin sodium-monosulfonate in 50 ml. distilled water and pour slowly into the zirconium oxychloride solution, while swirling the flask. This solution clears on standing for a few minutes.

Prepare a mixed acid solution as follows: Dilute 112 ml. of cp.  $\text{HCl}$  (Sp. gr. 1.19) to 500 ml. with distilled water. Add 37 ml. cp.  $\text{H}_2\text{SO}_4$  to 400 ml. distilled water and dilute to 500 ml. After cooling, mix the two acids. To the zirconium alizarin solution in the 1 liter flask, add the mixed acid solution to the mark and mix. The reagent changes in color from red to yellow within an hour and is then ready for use. It is fairly stable, and if stored in a refrigerator, may be used over a period of 60 to 90 days.

1.2. Standard sodium fluoride solution. Dissolve 0.221 g. cp.  $\text{NaF}$  in distilled water and make up to 1 liter. Dilute 100 ml. of this stock  $\text{NaF}$  soln. to 1 liter with distilled water. One ml. is equivalent to 0.01 mg.  $\text{F}^-$ .

#### 2. Procedure

To 100 ml. of water sample and to standards made up to 100 ml. with distilled water, contained in 100 ml. matched Nessler tubes, add 5 ml. of acid zirconium alizarin reagent, accurately measured from a 5 ml. volumetric pipette.

Mix and compare sample with standards after standing one hour at room temperature. Recommended

standards are 0, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.08, 0.10, 0.12, 0.14 mg. of  $F^-$ . If the fluoride content of the sample exceeds 1.4 ppm. repeat with an aliquot made up to 100 ml. with distilled water.

Since the color of the zirconium alizarin lake varies with temperature, samples and standards should have the same temperature within  $2^\circ C$ . before adding the reagents.

## B. WITH DISTILLATION

When any of the constituents listed in A, above are in excess of the indicated limits, equivalent quantities may be added to the standards in A, or the fluoride separated by distillation. The latter method is recommended as giving greater uniformity of procedure with unknown samples as it eliminates the necessity for complete analysis and has the advantage of eliminating unsuspected as well as known interference.

### 1. Reagents

1.1. Perchloric acid, 60 per cent.

1.2. Silver perchlorate solution. 5.84 g. of the salt dissolved in 100 ml. of distilled water. One ml. is equivalent to 10 mg. of  $Cl^-$ .

1.3. Silver sulfate. The solid salt.

### 2. Procedure

A 250 ml. sample of the water is made alkaline to phenolphthalein with NaOH and transferred to a separatory funnel. The separatory funnel is connected by means of rubber tubing to a glass tube extending through a one-hole rubber stopper to the bottom of the 200 ml. or 250 ml. round

bottom flask to be used for the subsequent distillation.

Portions of the sample from the separatory funnel are added from time to time to the flask as evaporation proceeds to a final volume of approximately 15 ml., or addition may be continuous as described below. The sample must be alkaline to phenolphthalein during the entire concentration process.

The concentrated solution is cooled, made slightly acid by the dropwise addition of 60 per cent perchloric acid and sufficient silver perchlorate solution added to precipitate the chloride ion present. This step may be omitted if the sample is known to contain less than 70 ppm. of  $Cl^-$ .

About a dozen glass beads are placed in the flask, 25 ml. of 60 per cent perchloric acid added, and the flask fitted with a three-hole rubber stopper carrying a thermometer and a capillary inlet tube whose inside diameter is between 0.4 mm. and 1.0 mm. and the condenser connected.

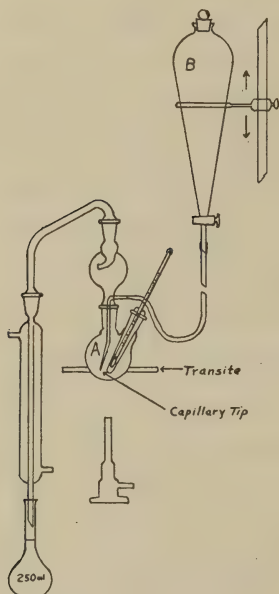
Both the thermometer and the inlet tube should extend below the surface of the solution and almost to the bottom of the flask.

The flask is placed in a perfectly fitting hole in an asbestos mat or board 8 in. square in such a manner that only that portion of the flask completely filled with solution projects below the mat. It is then connected to a water condenser, the temperature raised to  $135^\circ C$ . and held between  $135^\circ C$ . and  $138^\circ C$ . by the continuous addition of distilled water while collecting 250 ml. of distillate at the rate of about 4 ml. per minute.

The capillary glass water inlet tube

is connected by means of rubber tubing to a separatory funnel containing distilled water and by raising or lowering the separatory funnel with stopcock fully open, the rate of addition of distilled water may be made to approximate closely the rate of distillation.

The distillate is thoroughly mixed and the fluoride ion in a 100 ml. por-



FLUORIDE DISTILLATION ASSEMBLY

FIG. 5.

tion is determined by the method outlined in A above. For samples whose approximate fluoride content is unknown and for samples whose fluoride content is known to exceed 1.4 ppm., several different smaller aliquots may be made up to 100 ml. with distilled water and used for the determination and that tube read whose color falls within the range of the prepared standards.

A set-up almost identical with that shown in Fig. 5 with interchangeable ground glass joints, and requiring only the addition of the capillary glass tip, may be assembled from equipment manufactured by The Scientific Glass Apparatus Company, Bloomfield, N. J., or by Ace Glass, Inc., Vineland, N. J.

In the case of samples high in organic matter, the use of hot concentrated perchloric acid for the distillation constitutes a possible hazard. For such waters 15 ml. of concentrated sulfuric acid should be substituted for the perchloric acid and chloride precipitation, if required, accomplished by adding the necessary quantity of solid silver sulfate. If sulfuric acid is used, the distillation temperature may be allowed to reach, but not exceed, 145° C.

If desired, steam distillation may be employed. If this is done, the capillary water inlet tube is replaced by a steam inlet tube reaching almost to the bottom of the distillation flask. This tube is connected to an auxiliary boiling flask fitted with a two-hole rubber stopper and a glass tube dipping below the water surface and extending about 3 feet above the rubber stopper for pressure control.

#### BIBLIOGRAPHY

- DEBOER, H. H., AND BASART, J. A. A rapid volumetric determination of fluorine. *Zeit. fur Anorg. u. Allg. Chem.*, 152, 213 (1926).
- WILLARD, H. H., AND WINTER, O. B. Volumetric method for determination of fluorine. *Ind. Eng. Chem., Anal. Ed.*, 5, 7 (1933).
- THOMPSON, T. G., AND TAYLOR, H. J. Determination and occurrence of fluorides in sea water. *Ind. Eng. Chem., Anal. Ed.*, 5, 87 (1933).



SANCHIS, J. M. Determination of fluorides in natural water. *Ind. Eng. Chem., Anal. Ed.*, 6, 134 (1934).

DAHLE, D., AND WICHMANN, H. J. A quantitative study of fluorine distillation. *J. Assn. Off. Agr. Chem.*, 19, 313 (1936).

EBERZ, W. F., LABG, F. C., AND LACHELE, C. E. Determination of fluorine spray residue on tomatoes. *Ind. Eng. Chem., Anal. Ed.*, 10, 259 (1938).

COMMITTEE REPORT, Methods of determining fluorides. *J. Amer. W. W. Assn.*, 33, 1965 (1941).

SCOTT, R. D. Modification of the fluoride determination. *J. Amer. W. W. Assn.*, 33, 2018 (1941).

#### 40. Orthophosphate

##### A. GRAVIMETRIC

Ammonium molybdate precipitates phosphate as ammonium phosphomolybdate in nitric acid solution. The precipitate is dissolved and reprecipitated by magnesia mixture as magnesium ammonium phosphate in alkaline solution. The sample should contain between 4 and 40 mg. of phosphate as  $P_2O_5$ .

##### 1. Reagents

1.1. Nitric acid solution. Dissolve 250 g. of  $NH_4NO_3$  in 175 ml. of distilled water and 175 ml. of concd.  $HNO_3$ , and dilute to 500 ml. with distilled water.

1.2. Ammonium molybdate solution. Dissolve 75 g. of  $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$  and 75 g. of  $NH_4NO_3$  in 900 ml. of distilled water. Cool, add 60 ml. of concd.  $NH_4OH$  and dilute to 1 liter. Filter the solution before using.

1.3. Magnesium chloride solution. Dissolve 55 g. of  $MgCl_2 \cdot 6H_2O$  in a small volume of distilled water. Add 280 g. of  $NH_4Cl$ , 700 ml. of concd.  $NH_4OH$  and dilute to 1 liter with distilled water.

1.4. Ammonium hydroxide, 1:1.

1.5. Ammonium hydroxide, 1:4.

1.6. Hydrochloric acid, 1:1.

1.7. Nitric acid, 1:100.

##### 2. Procedure

To a 100 ml. clear sample, add 20 ml. of nitric acid solution and heat to  $80^\circ C$ . Add 15 ml. of  $(NH_4)_6Mo_7O_{24}$  soln. and stir until precipitation begins. Digest the precipitate for 1 hour at a temperature of  $40^\circ C$ . Filter and wash the precipitate with 1:100  $HNO_3$ .

Dissolve the precipitate with warm 1:1  $NH_4OH$ . Add 1:1  $HCl$  until the yellow precipitate which appears dissolves with difficulty. Add dropwise with stirring 10 ml. of  $MgCl_2$  soln. Add with stirring 25 ml. of concd.  $NH_4OH$ . Settle the precipitate for 2 hours. Filter and wash with 1:4  $NH_4OH$ . Ignite the precipitate carefully.

ppm.  $PO_4^{3-} = [mg. Mg_2P_2O_7 \times 853.4] \div ml. sample$ .

##### B. COLORIMETRIC—AMINO-NAPHTHOL-SULFONIC ACID METHOD

In a dilute phosphate solution, the addition of ammonium molybdate produces a colored phosphomolybdate which in turn forms a blue complex compound upon addition of a reducing agent such as amino-naphthol-sulfonic acid.

##### 1. Apparatus

One of the following pieces of equipment is required.

1.1. Nessler tubes for visual color comparison.

1.2. Duboseq type colorimeter for visual color comparison.

1.3. Photoelectric filter photometer.

1.4. Photoelectric spectrophotometer.

## 2. Reagents

2.1. Potassium phosphate standard solution. Dissolve 7.1638 g. of dried  $\text{KH}_2\text{PO}_4$  in 1 liter of distilled water. Dilute 10 ml. of the solution to 1 liter. One ml. of this solution contains 0.05 mg.  $\text{PO}_4^{=}$ .

2.2. Ammonium molybdate solution. Dissolve 25 g. of  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$  in 200 ml. of distilled water. Add 500 ml. of 1:3  $\text{H}_2\text{SO}_4$  and dilute to 1 liter with distilled water.

2.3. Sulfuric acid, 1:3.

2.4. 1-amino, 2-naphthol, 4-sulfonic acid solution. Grind 0.5 g. 1-amino, 2-naphthol, 4-sulfonic acid, take up with 5 ml. of sodium sulfite solution (20 g.  $\text{Na}_2\text{SO}_3$  per 100 ml.) and dissolve in 195 ml. of sodium metabisulfite solution (150 g.  $\text{Na}_2\text{S}_2\text{O}_5$  per liter). Do not use an amount of sodium sulfite greater than necessary to get the amino-acid in solution since it makes the solution less stable. Heat the solution, if necessary, to  $50^\circ\text{--}60^\circ\text{C}$ . to hasten dissolving. Keep the solution in a dark stoppered bottle and prepare fresh every two weeks. Use the purified grade of the amino-acid since the technical quality will not give satisfactory results.

## 3. Procedure

To a 50 ml. sample containing not more than 40 ppm. of  $\text{PO}_4^{=}$ , and free from color and turbidity, add one drop of phenolphthalein indicator and sufficient 1:3  $\text{H}_2\text{SO}_4$  to discharge the

indicator color. Then add 1 ml. more of 1:3  $\text{H}_2\text{SO}_4$ .

Dilute to approximately 70 ml. with distilled water. Add, mixing after each reagent addition, 10 ml. of  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$  soln. and 4 ml. of amino-acid solution. Dilute to 100 ml. with distilled water. After five minutes, compare colorimetrically against standards prepared simultaneously if using apparatus 1.1 or 1.2; or against a blank if using apparatus 1.3 or 1.4.

## C. COLORIMETRIC—STANNOUS CHLORIDE METHOD

In a dilute phosphate solution, the addition of ammonium molybdate produces a colored phosphomolybdate which in turn forms a blue complex compound on addition of a reducing agent such as stannous chloride.

### 1. Apparatus

One of the following pieces of equipment is required.

1.1. Nessler tubes for visual color comparison.

1.2. Duboseq type colorimeter for visual color comparison.

1.3. Photoelectric filter photometer.

1.4. Photoelectric spectrophotometer.

### 2. Reagents

2.1. Ammonium molybdate solution. Dissolve 25 g. of  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$  in approximately 175 ml. water. Dilute 280 ml. concd.  $\text{H}_2\text{SO}_4$  to 800 ml. Add the molybdate solution to the  $\text{H}_2\text{SO}_4$  soln. and dilute to 1 liter.

2.2. Stannous chloride solution. Dissolve 25 g. of  $\text{SnCl}_2$  in 1 liter of 1:9  $\text{HCl}$ . Filter, if not clear.

2.3. Potassium phosphate standard solution. Dissolve 7.1638 g. of dried  $\text{KH}_2\text{PO}_4$  in 1 liter of distilled water. Dilute 10 ml. of the solution to 1 liter. One ml. of this solution contains 0.05 mg  $\text{PO}_4^{=}$ .

### 3. Procedure

To a 50 ml. sample, free from color and turbidity, add, with mixing, 2 ml. of  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$  soln. and 0.25 ml. of stannous chloride solution. After five minutes, compare colorimetrically against standards prepared simultaneously if using apparatus 1.1 or 1.2; or against a blank if using apparatus 1.3 or 1.4.

### BIBLIOGRAPHY

- SCOTT, W. W. *Standard Methods of Chemical Analysis*, 4th Ed., p. 367. Van Nostrand, New York (1927).
- TRUOG, E., AND MEYER, A. H. Improvements in the Deniges colorimetric method for phosphorus and arsenic. *Ind. Eng. Chem., Anal. Ed.*, 1, 136 (1929).
- Joint Research Committee on Boiler Feed-water Studies, Report 1 (1932).
- SCHROEDER, W. C., AND FELLOWS, C. H. Determination of carbonate, hydroxide and phosphate in boiler waters. *Trans. A. S. M. E.*, 54, 213 (1932).
- PARTRIDGE, E. P. Determination of carbonate, phosphate, and hydroxide in boiled water. *A. S. M. E. Res. Publ.*, 1933.
- AM. SOC. TEST. MTLs. Method for determination of the total orthophosphate and calculation of the respective orthophosphate ions in industrial waters. A. S. T. M. Method D. 515-43, *A. S. T. M. Suppl. III*, 137 (1943).

## 41. Pyrophosphate

### A. MANGANOUS CHLORIDE SEPARATION METHOD

After removing metaphosphate from solution by barium chloride, the addition of manganous chloride precipitates pyrophosphate. The pre-

cipitate is dissolved by nitric acid and the pyrophosphate is converted to the orthophosphate by concentrated nitric acid. The orthophosphate is then determined by one of the methods outlined under Orthophosphate (Part I, Sec. 40, page 79).

### 1. Reagents

- 1.1. Manganous chloride solution. Dissolve 100 g. of  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  in 1 liter of distilled water.
- 1.2. Barium chloride solution. Dissolve 25 g. of  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$  in 1 liter of distilled water.
- 1.3. Hydrochloric acid, 1:11.
- 1.4. Methyl orange indicator solution.
- 1.5. Nitric acid, 1:1.
- 1.6. Sodium hydroxide, 0.1 N.
- 1.7. Acetone.
- 1.8. Barium chloride wash solution, 1.0 g. per liter.
- 1.9. Manganous chloride wash solution, 1.0 g. per liter.

### 2. Procedure

If necessary, dilute an aliquot of the clear sample, containing not more than 65 mg. of  $\text{PO}_4^{=}$ , to 50 ml. with distilled water. Add one drop of methyl orange indicator, acidify with 1:11 HCl to the end-point of methyl orange, then add 0.5 ml. of the acid in excess. Add slowly with constant stirring, 15 ml. of  $\text{BaCl}_2$  soln. and let the precipitate settle before filtering. In the absence of metaphosphate, omit the  $\text{BaCl}_2$  soln.

Filter off the precipitate and wash with a cold wash soln. of  $\text{BaCl}_2$ . Dilute the filtrate to 125 ml. with distilled water and add 5 ml. of  $\text{MnCl}_2$  soln.



Adjust the pH of the solution with the aid of a glass electrode or other suitable pH meter to 4.1 by adding 0.1 N NaOH drop by drop with stirring. Add with stirring 7 to 8 ml. of acetone and allow to stand 12 to 16 hours, if possible, for complete precipitation.

Filter off the precipitate and wash with a dilute  $\text{MnCl}_2$  wash soln. Dissolve the precipitate in 1:1  $\text{HNO}_3$ . Filter off any precipitate of barium sulfate remaining. Add 15 ml. of coned.  $\text{HNO}_3$  and boil the solution for 15 minutes.

Cool and neutralize the solution with  $\text{NH}_4\text{OH}$  so that it is just acid to litmus. Determine the phosphate present by one of the methods outlined under Orthophosphate (Part I, Sec. 40, page 79).

## B. DIFFERENCE METHOD

Pyrophosphate can be obtained by the difference between the phosphate content of two samples: (1)—untreated—giving only orthophosphate; (2) treated with concentrated nitric acid to convert pyrophosphate to orthophosphate—giving the sum of pyro- and ortho-phosphates. It should be noted that other forms of phosphate are also converted to orthophosphate by the acid treatment.

### 1. Reagents

- 1.1. Hydrochloric acid, 1:11.
- 1.2. Methyl orange indicator solution.
- 1.3. Nitric acid, 1:1.

### 2. Procedure

Add one drop of methyl orange indicator to 50 ml. of clear sample.

Acidify with 1:11 HCl to the end-point of methyl orange. Add 15 ml. of coned.  $\text{HNO}_3$  and boil the solution for 15 minutes. Cool and neutralize the solution with  $\text{NH}_4\text{OH}$  so that it is just acid to litmus.

Dilute the sample to 100 ml. with distilled water. Determine the phosphate present by Method C under Orthophosphate (Part I, Sec. 40, page 80), using a 100 ml. sample instead of the 50 ml. sample specified and increasing the quantities of reagents used proportionately.

## BIBLIOGRAPHY

JONES, L. T. Estimation of ortho-, pyro-, meta- and poly-phosphates in the presence of one another. *Ind. Eng. Chem., Anal. Ed.*, 14, 536 (1942).

## 42. Metaphosphate

### A. BARIUM CHLORIDE SEPARATION METHOD

Barium chloride precipitates metaphosphate from solution. The precipitate is dissolved by nitric acid and the metaphosphate is converted to the orthophosphate by concentrated nitric acid. The orthophosphate is then determined by one of the methods outlined under Orthophosphate (Part I, Sec. 40, page 79).

### 1. Reagents

- 1.1. Barium chloride solution. Dissolve 25 g. of  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$  in 1 liter of distilled water.
- 1.2. Hydrochloric acid, 1:11.
- 1.3. Methyl orange indicator solution.
- 1.4. Nitric acid, 1:1.
- 1.5. Barium chloride wash solution, 1.0 g. per liter.

## 2. Procedure

If necessary, dilute an aliquot of the clear sample, containing not more than 65 mg. of  $\text{PO}_4^{=}$ , to 50 ml. with distilled water. Add one drop of methyl orange indicator, acidify with 1:11 HCl to the end-point of methyl orange, then add 0.5 ml. of the acid in excess.

Add slowly with constant stirring 15 ml. of  $\text{BaCl}_2$  soln. and let the precipitate settle before filtering. Wash the precipitate by decantation with a small amount of cold  $\text{BaCl}_2$  wash soln.

Filter and wash the precipitate with the same solution four times. Dissolve the precipitate in 1:1  $\text{HNO}_3$ . Filter off any precipitate of barium sulfate remaining. Add 15 ml. of concd.  $\text{HNO}_3$  and boil the solution for 15 minutes.

Cool and neutralize the solution with  $\text{NH}_4\text{OH}$  so that it is just acid to litmus. Determine the phosphate present by one of the methods outlined under Orthophosphate (Part I, Sec. 40, page 79).

## B. DIFFERENCE METHOD

Metaphosphate can be obtained by the difference between the phosphate content of two samples: (1) untreated—giving only orthophosphate; (2) treated with concentrated nitric acid to convert metaphosphate to orthophosphate—giving the sum of meta- and ortho-phosphates. It should be noted that other forms of phosphate are also converted to orthophosphate by the acid treatment.

### 1. Reagents

1.1. Hydrochloric acid, 1:11.

1.2. Methyl orange indicator solution.

1.3. Nitric acid, 1:1.

## 2. Procedure

Add one drop of methyl orange indicator to 50 ml. of clear sample. Acidify with 1:11 HCl to the end-point of methyl orange. Add 15 ml. of concd.  $\text{HNO}_3$  and boil the solution for 15 minutes.

Cool and neutralize the solution with  $\text{NH}_4\text{OH}$  so that it is just acid to litmus. Dilute the sample to 100 ml. with distilled water.

Determine the phosphate present by Method C under Orthophosphate (Part I, Sec. 40, page 80), using a 100 ml. sample instead of the 50 ml. sample specified and increasing the quantities of reagents used proportionately.

## BIBLIOGRAPHY

JONES, L. T. Estimation of of ortho-, pyro-, meta-, and poly-phosphates in the presence of one another. *Ind. Eng. Chem., Anal. Ed.*, 14, 536 (1942).

## 43. Sulfate

### A. GRAVIMETRIC

In an acid solution barium chloride precipitates only sulfates as barium sulfate. Sulfites and large amounts of silica and iron should be absent. The gravimetric method is recognized as the primary standard procedure.

### 1. Reagents

1.1. Barium chloride solution. Dissolve 10 g. of  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$  in 100 ml. of distilled water.

1.2. Hydrochloric acid, 1:1.

1.3. Silver nitrate-nitric acid wash solution. Dissolve 8.5 g. of  $\text{AgNO}_3$

and 0.5 ml. of concd.  $\text{HNO}_3$  in 500 ml. of distilled water.

## 2. Procedure

Add 2 ml. of 1:1  $\text{HCl}$  to a clear 250 ml. sample, adjusted by dilution or concentration to contain approximately 50 mg. of  $\text{SO}_4^{--}$ . Heat the solution to boiling and add slowly, with stirring, a slight excess of hot  $\text{BaCl}_2$  soln.

Digest the precipitate on a steam bath for 2 hours. Filter and wash the precipitate with warm distilled water until chloride-free as determined by use of the silver nitrate-nitric acid wash solution. Ignite the precipitate at  $800^\circ \text{C}$ . to constant weight.

$\text{ppm. SO}_4^{--} = [\text{mg. of BaSO}_4 \times 411.4] \div \text{ml. sample.}$

## B. BENZIDINE METHOD

In acid solution, benzidine hydrochloride precipitates sulfates as benzidine sulfate. The precipitate hydrolyzes to form sulfuric acid in amount equivalent to the sulfates originally present. The sulfuric acid is titrated with standard sodium hydroxide. The solubility of benzidine sulfate in water is significant. The procedure attempts to minimize this effect.

### 1. Reagents

1.1. Benzidine hydrochloride solution. Dissolve 11.2 g. of benzidine hydrochloride in 400 ml. of distilled water, and mix with 100 ml. of 1:8  $\text{HCl}$ . Saturate this solution with benzidine sulfate and filter.

1.2. Phenolphthalein indicator solution.

1.3. Sodium hydroxide solution, 0.1 N. One ml. of this solution is equivalent to 4.803 mg.  $\text{SO}_4^{--}$ .

1.4. Wash water solution. Saturate distilled water with benzidine sulfate, then filter.

## 2. Procedure

Add a clear sample, of suitable size, to a 250 ml. beaker or Erlenmeyer flask. Use a 50 ml. sample if the  $\text{SO}_4^{--}$  concentration is between 135 and 1000 ppm. The sample should be adjusted either by dilution or by evaporation to fall within these limits. Add 10 ml. of the benzidine solution.

Thoroughly mix the solution by stirring or by giving the flask a whirling motion, then allow the mixture to stand for ten minutes. Filter off the benzidine sulfate precipitate through quantitative filter paper pulp. Test the filtrate for the presence of unprecipitated sulfates by adding a few drops of benzidine hydrochloride solution. Filter off any additional precipitate which may form.

Rinse the emptied beaker or flask and wash the precipitate with approximately 25 ml. of distilled water, saturated with benzidine sulfate. Do this in three separate portions, allowing each portion to drain completely. Discard the rinse water. Remove the filter paper pulp and precipitate. Rinse out the filter crucible with approximately 25 ml. of distilled water and place it in the original container.

Add several drops of phenolphthalein indicator solution. Titrate with 0.1 N  $\text{NaOH}$ , shaking vigorously after each addition of base, until a pink color results which lasts for half a minute.



Obtain a blank correction by following the procedure exactly, using the same volume of distilled water as the original sample. Record the ml. of 0.1 N NaOH required to obtain an end point.

$\text{ppm. SO}_4^{2-} = [(\text{ml. NaOH for sample} - \text{ml. NaOH for blank}) \times 4800] \div \text{ml. sample.}$

$\text{ppm. SO}_4^{2-} \text{ as Na}_2\text{SO}_4 = [(\text{ml. NaOH for sample} - \text{ml. NaOH for blank}) \times 7100] \div \text{ml. sample.}$

#### C. TETRAHYDROXYQUINONE METHOD

Barium chloride precipitates sulfates as barium sulfate. Further addition of barium compound produces a color change from yellow to red due to the presence of an internal indicator, disodium tetrahydroxyquinone. Phosphates interfere, therefore a special procedure is followed when they are present. For best results, the sulfate concentration should be over 100 ppm.

##### 1. Reagents

1.1 Barium chloride solution, 0.025 N. Dissolve 3.054 g. of  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$  in one liter of distilled water. Standardize by the Gravimetric Method (Part I, Sec. 43, A, page 83).

1.2 Tetrahydroxyquinone indicator.

1.3 Ethyl or isopropyl alcohol.

1.4 Silver nitrate solution. Dissolve 2 g. of  $\text{AgNO}_3$  in 100 ml. of distilled water.

1.5 Phenolphthalein indicator solution.

1.6 Brom cresol green indicator solution.

1.7 Hydrochloric acid, 1:23.

##### 2. Procedure

2.1. Phosphate absent. Use a 25 ml. clear sample containing more than 100 ppm. of sulfate. Add 1 ml. of 1:23 HCl and boil only if sulfites are present. Add several drops of phenolphthalein indicator solution to the sample in an Erlenmeyer flask. Adjust either with dilute acid or base until the sample is just colorless to the indicator.

Add 25 ml. of ethyl or isopropyl alcohol. Add 0.21 g. of tetrahydroxyquinone indicator. Titrate the solution with 0.025 N  $\text{BaCl}_2$  with thorough stirring, until the yellow color of the solution changes to red.

Obtain a blank correction by following the procedure exactly, using the same volume of distilled water as the original sample. Record the volume of 0.025 N  $\text{BaCl}_2$  required to obtain an end-point.

The sharpness of the end point is increased by the addition of a small amount of silver nitrate. Before titrating with  $\text{BaCl}_2$  soln., add to the sample 1 to 3 ml. of  $\text{AgNO}_3$  soln., depending on the concentration of chlorides present. An excess of silver nitrate will produce an intense red color. If this occurs, discard the sample and repeat using a smaller amount of silver nitrate solution.

2.2. Phosphate present. If phosphates are present, add several drops of brom cresol green indicator solution instead of phenolphthalein indicator solution and neutralize the sample until the green color of the indicator turns a straw color. Boil only if sulfites are present.

Add 25 ml. of ethyl or isopropyl alcohol. Add 0.21 g. of tetrahydroxy-

quinone indicator. Titrate the solution with 0.025 N  $\text{BaCl}_2$  with thorough stirring, until the yellow color of the solution changes to red.

Obtain a blank correction by following the procedure exactly, using the same volume of distilled water as the original sample. In this case, an amount of phosphate, approximately equivalent to that present in the sample, should be added to the distilled water. Record the volume of 0.025 N  $\text{BaCl}_2$  required to obtain an end-point.

The sharpness of the end-point is increased by the addition of a small amount of silver nitrate. Before titrating with  $\text{BaCl}_2$  soln., add to the sample 1 to 3 ml. of  $\text{AgNO}_3$  soln., depending on the concentration of chlorides present. An excess of silver nitrate will produce an intense red color. If this occurs, discard the sample and repeat using a smaller amount of silver nitrate solution.

$\text{ppm. SO}_4 = [(\text{ml. BaCl}_2 \text{ for sample} - \text{ml. BaCl}_2) \times 1200] \div \text{ml. sample.}$

$\text{ppm. SO}_4 \text{ as Na}_2\text{SO}_4 = [(\text{ml. BaCl}_2 \text{ for sample} - \text{ml. BaCl}_2 \text{ for blank}) \times 1776] \div \text{ml. sample.}$

## BIBLIOGRAPHY

JOHNSTON, J., AND ADAMS, L. H. The phenomenon of occlusion in precipitates of barium sulfate and its relation to the exact determination of sulfate. *J. Am. Chem. Soc.*, **33**, 829 (1911).

PARR, S. W. *Fuel, Gas, Water and Lubricants*, p. 261, McGraw-Hill, New York (1932).

SCHROEDER, W. C. Determination of sulfate by a direct titration using tetrahydroxyquinone. *Joint Research Committee on Boiler Feed Water Studies Report*, January, 1933.

SCHROEDER, W. C. Direct titration for sulfate. *Ind. Eng. Chem., Anal. Ed.*, **5**, 6, 403 (1933).

SHEEN, R. T., AND KAHLER, W. H. Direct titration of sulfates. *Ind. Eng. Chem., Anal. Ed.*, **8**, 127 (1936).

KAHLER, H. L., BETZ, W. H., AND BETZ, L. D. Determining sulfate by the tetrahydroxyquinone method. Effect of sodium sulfite and procedure for its elimination. *Ind. Eng. Chem., Anal. Ed.*, **12**, 266 (1940).

RIEMAN, W., AND HAGEN, G. Determination of sulfur. *Ind. Eng. Chem., Anal. Ed.*, **14**, 150 (1942).

AM. SOC. TEST. MTLs. Method for determination of the sulfate ion in industrial water. Method D 516-42, *A. S. T. M. Suppl. III*, 137 (1943).

## 44. Sulfite

### A. VOLUMETRIC

Upon addition of a measured quantity of sample to an excess of iodine solution, the sulfite present reduces the iodine. The excess remaining is determined iodimetrically with sodium thiosulfate, using starch as an internal indicator.

### 1. Reagents

1.1. Glacial acetic acid.

1.2. Iodine solution, 0.025 N.

1.3. Sodium thiosulfate standard solution, 0.025 N.

1.4. Starch indicator solution.

### 2. Procedure

From a pipette, measure 10 ml. of 0.025 N iodine solution and 5 ml. of glacial  $\text{CH}_3\text{COOH}$  into a small mouthed flask or clean white casserole. Then add slowly with stirring 100 ml. of sample avoiding exposure to the air.

To this mixture, while stirring with a glass rod, quickly add 0.025 N  $\text{Na}_2\text{S}_2\text{O}_3$  soln. from a burette until the brown color of the iodine is almost gone. Then add 2 ml. of starch indicator solution and continue to add the

$\text{Na}_2\text{S}_2\text{O}_3$  until the blue coloration, due to the starch iodide, disappears.

Repeat the foregoing procedure using 100 ml. of water, free from sulfite, in place of the sample to obtain a blank reading.

$\text{ppm. Na}_2\text{SO}_3 = (\text{ml. Na}_2\text{S}_2\text{O}_3 \text{ for sample} - \text{ml. Na}_2\text{S}_2\text{O}_3 \text{ for blank}) \times 15.8.$

#### 45. Sulfides

Methods for the determination of sulfides are described in Part II, Sec. 20, page 152.

##### A. SAMPLING

Water containing dissolved sulfides readily loses hydrogen sulfide, particularly if the pH of the sample is low. Oxygen destroys sulfide by oxidation, particularly if the pH of the sample is high. Aeration of the samples should therefore be avoided. It is advisable to fix the sample by addition of a suitable quantity of zinc acetate solution which precipitates the sulfide as zinc sulfide. In case the sample is not fixed in this manner, it should be analyzed immediately.

##### B. TOTAL SULFIDES BY EVOLUTION METHOD

Total sulfides include dissolved and suspended sulfides. In acid solution sulfides liberate hydrogen sulfide which is removed by scrubbing with carbon dioxide and then absorbed in zinc acetate solution. The precipitated sulfide is titrated iodimetrically in acid solution using starch as an indicator.

The procedure is described in Part II, Sec. 20, B, 2.1, page 153.

##### C. DISSOLVED SULFIDES BY EVOLUTION METHOD

Dissolved sulfides are determined by the same procedure as that used for total sulfides after the suspended solids have been removed by flocculation and settling.

The procedure is described in Part II, Sec. 20, B, 2.2, page 153.

##### D. DISSOLVED SULFIDES BY VOLUMETRIC METHOD

In the absence of suspended matter, dissolved sulfides may be determined by direct precipitation with zinc acetate followed by iodimetric titration in acid solution using starch as an indicator.

##### E. TOTAL AND DISSOLVED SULFIDES BY COLORIMETRIC METHOD

In dilute sulfide solutions, up to 20 ppm., p-aminodimethylaniline, ferric ion and chloride ion produce methylene blue which can then be determined by suitable colorimetric apparatus.

The procedure is described in Part II, Sec. 20, C, 2.1-2.2, page 154.

#### 46. Boron

The addition of mannitol to a neutral, unbuffered solution of mixed salts containing boron causes the solution to become acid. The quantity of standard alkali required to titrate the solution back to the initial pH is an accurate measure of the boron present. Electrometric or direct methods of titration may be used, the former being described below,



## 1. Apparatus

The choice of apparatus for the electrometric titration of boron should be determined by the instruments available, the number of analyses to be made and the frequency of use. Three sets of apparatus are described below, any one of which will give satisfactory results. The first requires a minimum of equipment. The operation depends on the fact that a 0.7 N calomel electrode, and a quinhydrone electrode come to a null point (reversal of polarity) at approximately pH 7.0.

1.1. Galvanometer. An enclosed lamp and scale type sensitive to 0.025 microampere per scale division.

1.2. Quinhydrone electrode. A piece of platinum wire 7.5 cm. (3 inches) in length, with suitable contact above the surface of the solution. This type is preferable to an electrode of platinum sealed through glass and connected with mercury, as minute cracks develop in the glass and cause erratic results.

1.3. Calomel electrode, 0.7 N with respect to potassium chloride. A silver-silver chloride electrode can be used in place of the 0.7 N calomel electrode. For details see the original paper (Ref. Wilcox, 1932).

1.4. Motor stirrer.

1.5. Switch, single-pole single-throw.

The electrodes are connected through the switch to the galvanometer. A shunt to protect the galvanometer is desirable but not essential.

The second apparatus is a simple potentiometer the circuit diagram for which is shown in Fig. 6. In addition to the parts listed above, the following

are required: resistance wire, 1500 ohms tapped at 60 ohms; a 1.5 volt dry cell; and a calomel electrode, 0.1 N with respect to potassium chloride. This is substituted for the 0.7 N electrode, described above.

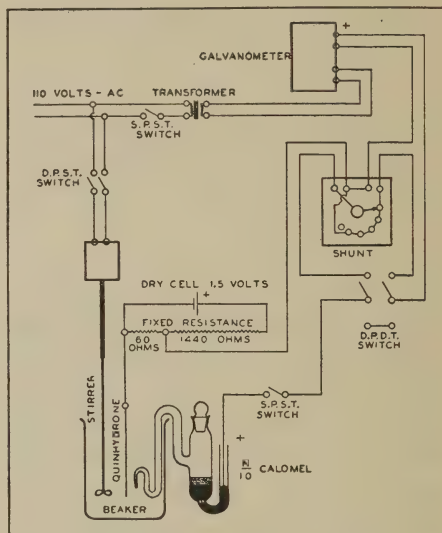


FIG. 6. CIRCUIT DIAGRAM OF THE ELECTROMETRIC TITRATION APPARATUS. THE 6 V A.C. LINE FROM THE TRANSFORMER SUPPLIES THE LIGHT IN THE REFLECTING GALVANOMETER.

The third apparatus makes use of either a potentiometer or a "pH meter" as the indicating system. The instrument is set so that, at balance, the solution under test will have a pH of 7.0. The following electrode pairs have been found satisfactory: quinhydrone and 0.1 N calomel; quinhydrone and saturated calomel; glass and saturated calomel.

## 2. Reagents

2.1. Quinhydrone, reagent quality, free from heavy metals.

2.2. Bromthymol blue indicator

solution, 1 per cent. Methyl red may be substituted.

2.3. Sulfuric acid. Approximately 1 N.

2.4. Sulfuric acid. Approximately 0.02 N.

2.5. Sodium hydroxide. Approximately 0.5 N carbonate-free.

2.6. Sodium hydroxide. Standard 0.0231 N, carbonate-free (1 ml. is equivalent to 0.25 mg. B).

2.7. Boric acid solution. Dissolve 0.5716 g. A.C.S. reagent grade, dry  $H_3BO_3$  in distilled water and dilute to 1 liter. One ml. contains 0.1 mg. B. This solution is used in standardizing the NaOH (2.6).

2.8. Mannitol, neutral. Synthetic mannitol is preferable to the natural product. The "blank" titration for 5 g. of mannitol should not exceed 0.1 ml. of the standard 0.0231 N NaOH (2.6).

### 3. Procedure

3.1. Preparation of sample. Transfer 250 ml. of the sample to a 400 ml. beaker. This should contain not over 1 mg. of elemental boron. If the sample is high in boron, an aliquot portion, diluted to 250 ml. should be taken.

Add a few drops of bromthymol blue (2.2) and acidify with 1 N  $H_2SO_4$  (2.3) adding 0.5 to 1 ml. in excess. Bring to boil, stir, cautiously at first, then vigorously, to expel carbon dioxide. Cool to room temperature, preferably in a water bath.

3.2. Electrometric titration. With the S.P.S.T. switch open, and the shunt, if used, set at 0.1, introduce the electrodes and stirrer into the solution. Start the stirrer and add

carbonate-free 0.5 N NaOH (2.5) to approximate neutrality as shown by the bromthymol blue.

Add about 0.2 g. quinhydrone (2.1). Close the switch in the electrode circuit. The galvanometer should indicate approximate balance. If it swings to the right, excess alkali is indicated, and if to the left, excess acid. Adjust with either 0.0231 N NaOH (2.6) or dil.  $H_2SO_4$  (2.4) until the galvanometer shows no deflection.

If a shunt is used, reverse the switch, thus eliminating it from the circuit and permitting the galvanometer to function at its greatest sensitivity. Again adjust to balance with either dilute acid or alkali. The galvanometer should be steady, showing at most only a slow drift. This is the initial point of the titration.

Bring the shunt into the circuit by reversing the D.P.D.T. switch or open the S.P.S.T. switch if the shunt is omitted. Add  $5 \pm 0.1$  g. of mannitol. If boron is present, the indicator will change to the acid color and the galvanometer will swing to the left. Add standard 0.0231 N NaOH (2.6) until approximate balance is again attained; eliminate the shunt, if used, and complete the titration bringing the galvanometer back to the original null point. This is the end point.

Note the number of ml. of standard NaOH (2.6) required after adding the mannitol at the initial point of the titration. From this, subtract a blank determined by substituting distilled water for the sample and proceeding as indicated above. The net volume of standard NaOH multiplied by the equivalency (mg. B per ml. NaOH)

gives mg. B in the aliquot titrated. Report as parts per million B.

The equivalency of the standard NaOH (2.6) is established by titrating an aliquot of the boric acid solution (2.7). The burette used should be of such accuracy that the volume of standard NaOH can be read to 0.01 ml. Borosilicate glassware (Pyrex) can be used for this determination. New beakers should be filled with acid and heated on the steam bath before use.

If the concentration of phosphate exceeds 10 ppm., it should be precipitated with lead nitrate and the excess lead removed with sodium bicarbonate.

ppm. B =  $\text{ml. NaOH} \times \text{mg. B equivalent to 1 ml. NaOH} \times 1000 \div \text{ml. sample}$ .

## BIBLIOGRAPHY

- WILCOX, L. V. Electrometric titration of boric acid. *Ind. Eng. Chem. Anal., Ed. 4*, 38-39 (1932).
- FOOTE, F. J. Determination of boron in waters. *Ind. Eng. Chem. Anal., Ed. 4*, 39 (1932).
- WILCOX, L. V. Determination of boron in plant material; *Chronica Botanica VI*, 16, 370-372 (1941).

## 47. Cyanide

### A. COLORIMETRIC

Yellow ammonium sulfide converts cyanide to thiocyanate in slightly alkaline solution. The thiocyanate reacts quantitatively with ferric ion to form colored ferric thiocyanate. The cyanide ( $\text{CN}^-$ ) concentration should be between 0.05 mg. and 2.0 mg. in 50 ml. of distillate or 0.1 to 4.0 ppm. in the original water. Color comparison may be made against standard solutions of cyanide or potassium thiocyanate.

### 1. Apparatus

1.1. Nessler tubes for visual color comparison.

1.2. Duboseq type colorimeter for visual color comparison.

### 2. Reagents

2.1. Yellow ammonium sulfide. Ordinary laboratory reagent.

2.2. Sodium hydroxide solution, 0.25 N.

2.3. Hydrochloric acid, 1:8.

2.4. Ferric chloride solution. Dissolve 10 g. of  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  in 100 ml. of distilled water.

2.5. Stock solution of potassium thiocyanate. Dissolve 4 g. of potassium thiocyanate in 1 liter of distilled water. Determine the exact strength of the solution by titration with 0.1 N  $\text{AgNO}_3$  and dilute with distilled water so that 1.0 ml. contains 1.0 mg.  $\text{CN}^-$  (1 ml. 0.1 N  $\text{AgNO}_3$  = 2.6 mg.  $\text{CN}^-$ ).

2.6. Dilute standard solution of potassium thiocyanate. Dilute 10 ml. of stock solution of KSCN (2.5) with distilled water to 100 ml. One ml. of this solution is equivalent to 0.1 mg.  $\text{CN}^-$ .

2.7. Tartaric acid cp.

### 3. Procedure

Acidify 500 ml. of the sample with 0.5 g. of tartaric acid, distill and collect 50 ml. of distillate. Place the whole distillate, or an aliquot containing less than 2.0 mg.  $\text{CN}^-$ , in an evaporating dish, add 0.2 ml. of 0.25 N NaOH and 0.5 ml. of yellow ammonium sulfide solution and evaporate just to dryness on the water bath.

If the yellow color should fade at any time during the evaporation, add a further drop or two of yellow ammo-



mium sulfide solution. Take up the residue with 10 ml. of distilled water, add 1.0 ml. of 1:8 hydrochloric acid and heat just to boiling.

Allow the mixture to stand several hours for the sulfur to coagulate, then filter and wash into a 50 ml. Nessler tube until about 40 ml. have passed through. Prepare a series of standard tubes containing from 0.5 ml. to 20 ml. dilute standard KSCN soln. corresponding to 0.05 mg. to 2.0 mg. cyanide. Dilute to about 40 ml. and acidify with 1.0 ml. of 1:8 hydrochloric acid.

To sample and standards, add 1.0 ml. of the  $\text{FeCl}_3$  soln. and adjust the volume of each tube to 50 ml. with distilled water. Mix and match the colors immediately.

If the amount of cyanide is above 0.1 mg., the test may be simplified by making fewer standards and comparing with the nearest one in a colorimeter.

#### BIBLIOGRAPHY

FASKEN, J. E. Determination of small amounts of cyanide in water. *J. Am. W. W. Assn.*, 32, 487 (1940).

#### 48. Tannin and Lignin

Tannins and lignins reduce phosphotungstic and phosphomolybdic acids to produce a blue color suitable for colorimetric and photometric estimation.

##### 1. Apparatus

One of the following pieces of equipment is required.

1.1. Nessler tubes for visual color comparison.

1.2. Duboseq type colorimeter for visual color comparison.

1.3. Photoelectric filter photometer.

1.4. Photoelectric spectrophotometer.

##### 2. Reagents

2.1. Tyrosine reagent. Dissolve 100 g. of sodium tungstate, 20 g. of phosphomolybdic acid and 50 ml. of 85 per cent phosphoric acid in 750 ml. of distilled water. Boil the liquid under reflux for 2 hours, cool and make up to 1 liter with distilled water.

2.2. Sodium carbonate solution, saturated. Keep this solution in a rubber-stoppered bottle.

2.3. Comparison solution. Dissolve 5.000 g. of the tannin or lignin compound being used in 1 liter of distilled water. Dilute 10 ml. of this solution to 1 liter with distilled water. Do not keep longer than 2 months. One ml. of solution = 0.05 mg. of compound.

2.4. Tannic acid standard solution. Dissolve 1.000 g. of chemically pure tannic acid in 1 liter of distilled water. Dilute 25 ml. of this solution to 1 liter with distilled water. One ml. of solution = 0.025 mg. of tannic acid.

##### 3. Procedure

Add 2 ml. of tyrosine reagent to 50 ml. of a clear sample containing not more than 3 ppm. of tannin or 20 ppm. of lignin. Mix, wait 5 minutes, then add 10 ml. of sodium carbonate solution and mix. Wait 10 minutes for color development.

Compare colorimetrically against comparison or standard solution prepared simultaneously if using appa-

ratus 1.1 or 1.2; or against a blank if using apparatus 1.3 or 1.4.

#### BIBLIOGRAPHY

BERK, A. A., AND SCHROEDER, W. C. Determination of tannin substances in boiler water. *Ind. Eng. Chem., Anal. Ed.*, 14, 456 (1942).

#### 49. Residual Chlorine

The procedures recommended in this and the following section (Chlorine Demand, Part I, Sec. 50, page 103) are based on a report of the A. W. W. A. Committee on Control of Chlorination.

Methods for the determination of free available chlorine or combined available chlorine are necessarily based on reactions with reducing agents that are not specific for these materials. Definite test conditions under which such specificity is attained in the presence of particular interfering oxidizing agents have been described by a number of investigators, and the results have been considered in formulating this section.

The purity of ortho-tolidine, as purchased on the market, has always been a matter of concern. Ortho-tolidine dihydrochloride, as sold on the open market, is very much purer than the free base. The salt has an advantage in that it is soluble in water, a characteristic which greatly facilitates preparation of the test solutions.

The concentration of the acid in the ortho-tolidine reagent must be such that it will produce a suitable pH in the sample under examination even if the sample has up to 1000 ppm. alkalinity.

To insure a pH of 1.3 or lower and a ratio of ortho-tolidine to chlorine

of at least 3:1 whenever the two are in contact, it is specified that the reagent be placed in the cell, tube or comparison bottle first and the sample added to it.

The six month time limit for replacement of ortho-tolidine reagent is an arbitrary precaution against the effect of possible occasional exposure to high temperatures or direct sunlight, resulting in discoloration or precipitation. The caution against contact with rubber is based on observations that significant amounts of reducing substances are extracted from some types of rubber closures by ortho-tolidine reagent.

The reaction time and the temperature specified were selected to permit measurement of a maximum proportion of the combined available chlorine present, while minimizing loss of color by fading or increase of color due to interfering oxidizing agents which react slowly with ortho-tolidine (nitrites, ferric iron and compounds formed by chlorine with organic matter).

The method given for preparation of zero-chlorine-demand water was selected for convenience; although ammonia-free water is not required, the preference expressed for it is based on difficulties encountered in removing large amounts of ammonia with chlorine alone. Due to the rapidity with which this water may absorb some laboratory fumes, it should be examined for nitrite, chlorine, or reducing agents immediately before use for special investigations dealing with very low concentrations of chlorine.

The modified Scott chromate-dichro-

mate color standards give good visual matches and almost exact spectrophotometric matches when the chlorine solution is at pH 1.3 and the ortho-tolidine: chlorine ratio at least 3:1. The choice of buffer solutions and their application are the result of spectrophotometric studies. When properly prepared they give satisfactory results.

The iodimetric method is used as a standard because it is the basis for the standardization of chlorine water used in preparing temporary standards. It is also suitable for determining high chlorine residuals, which are now more frequently encountered than heretofore.

The ortho-tolidine flash test is a qualitative technic for chlorine uncombined with ammonia, amines, or organic matter and is unaffected by the slow-acting interfering agents, nitrites, or oxidized iron, but is affected by colloidal manganese dioxide.

The ortho-tolidine-arsenite test is a technic for the differentiation of free available chlorine, combined available chlorine, and color due to interfering substances.

The methods given are intended for use: (a) on polluted water, (b) on water in process of purification, (c) at the end of the purification process, (d) incidental to the distribution of water prepared for human consumption, (e) on swimming pool water, (f) on condenser water and (g) on industrial process water.

#### A. ORTHO-TOLIDINE METHOD

When ortho-tolidine is used to measure residual chlorine the analyst must satisfy himself as to the presence and

the amount of interfering substances in the sample to be tested. Such substances include nitrites, ferric compounds, manganic compounds, and possibly, organic iron compounds, lignocellulose, and algae.

The effect of these substances is to increase the apparent residual chlorine content of the sample under examination.

Suspended matter interferes and should be removed by centrifuging prior to test or, if the turbidity is not too high, a compensating colorimeter, which also corrects for existing color and turbidity in the sample, may be used.

In chlorinated water containing no more than 0.3 ppm. iron, 0.01 ppm. manganic manganese and/or 0.10 ppm. of nitrite nitrogen, the development of the characteristic yellow color with ortho-tolidine may be accepted as being due to chlorine.

If iron and manganese are present in more than the above amounts, the development of the characteristic yellow color with ortho-tolidine may not be accepted as being due to chlorine alone.

If nitrite is present in an interfering amount, the provision that the color development with ortho-tolidine be carried on in total darkness minimizes such interference.

It should be noted that significant amounts of nitrite will not exist in water containing free available chlorine but may exist in the presence of chloramine.

It is recommended that the ortho-tolidine-arsenite test (Sec. 49, D, page 100) be employed to determine the amount of any interfering colors pro-



duced by the above substances. When such interfering colors are found, this test should be used as the routine procedure.

The ortho-tolidine method measures both the free available chlorine and that combined with nitrogenous compounds. If it is desired to determine whether the chlorine is present in free or combined form, the "flash test" (Sec. 49, C, page 100) or the ortho-tolidine-arsenite test (Sec. 49, D, page 100) may be used.

To obtain the correct color development with chlorine and ortho-tolidine up to 10 ppm., (a) the solution must be at pH 1.3 or lower during the contact period and (b) the ratio of ortho-tolidine to chlorine (both by weight) must be at least 3:1.

### 1. Apparatus—Light Sources

1.1. Illumination. All readings should be taken looking through the samples against an illuminated white surface. This white surface may be opaque and illuminated by reflection or may be an opal diffusing glass illuminated from behind. Since, in plant control, the chlorine determination is one which should be made both day and night, it is preferable that all comparisons be made with a standard artificial light. The permanent standards (A, 3.1–3.8) give greatest accuracy when used with either of the two artificial light sources specified (A, 1.3), both of which are close approximations of average "north" daylight.

1.2. Natural daylight. If artificial light is not provided, comparisons should be made with good "north" daylight. Under no circumstances

should the comparisons be made in the sunlight.

1.3. Artificial daylight. This may consist of filament or fluorescent "daylight" lamp assemblies.

1.3.1. Filament lamp assembly: This should consist of a light source, color filter and diffuser glass, suitably mounted in a cabinet. It is essential that the diffuser glass be placed in contact with the color filter and on the sample side. *The light source* should be a 150-watt Mazda "C" lamp, with clear envelope. *The color filter* should be Corning Glass "daylight" No. 5900, having a thickness equivalent to 163 micro-reciprocal degrees ( $\text{mr}^\circ$ ). *The diffuser glass* should be thin flashed opal glass without color tint.

1.3.2. Fluorescent "daylight" lamp assembly. The white-reflecting or diffusing surface may be illuminated by a fluorescent "daylight" lamp without the use of a color filter. Those manufactured by the General Electric Co. or the Westinghouse Electric and Mfg. Co. or equal should be specified.

### 2. Temporary Chlorine Standards

Temporary chlorine standards are not recommended for routine use, because extreme care is required in the preparation of these standards if the results are to be accurate; such care is seldom justified by the very temporary nature of the standards. These standards are recommended for calibration purposes and for research.

2.1. Zero-chlorine-demand water. Add sufficient chlorine to distilled water to destroy the ammonia. The amount of chlorine required will be

about 10 times the amount of  $\text{NH}_3\text{-N}$  present and in no case should the initial residual be less than 1.0 ppm. chlorine. Allow the chlorinated distilled water to stand overnight or longer; then expose to direct sunlight until all residual chlorine is discharged. The use of  $\text{NH}_3\text{-N}$  and  $\text{NO}_2\text{-N}$  free distilled water expedites obtaining a zero-chlorine-demand water.

2.2. Chlorine solution for temporary standards. Chlorine solutions or hypochlorite solutions for research and calibration purposes should be prepared from purified reagents and standardized as described below. Certain commercial solutions of hypochlorite have been found to be of relatively constant composition and high degree of stability. Because of their convenience, their use is preferred for routine purposes.\*

2.3. Preparation of standards. All glassware must be aged by being filled with water containing at least 10 ppm. chlorine for a few hours before use and then rinsed with zero-chlorine-demand water.

Add calculated volumes of hypochlorite solution to a series of flasks containing zero-chlorine-demand water to cover the range desired. Make up each one to 500 ml.

Pipette 100 ml. from each flask to a series of smaller flasks, each containing 5 ml. ortho-tolidine (A, 4.1). Add the chlorine solution slowly, mixing well as it is added. Pour the required amount into the comparator cell or tube. If 100 ml. Nessler tubes

are used, the second series of flasks may be omitted.

2.4. Titration of chlorine solution. Pour the remaining 400 ml. chlorine water (A, 2.3) into a white porcelain casserole and titrate iodometrically with acid (B, 3.1-3.3) unless amperometric equipment which is preferable, is available.

When the greatest accuracy is not required, suitable volumes of a chlorine solution, standardized iodometrically (B, 3.1-3.3), may be added to zero-chlorine-demand water containing 5 ml. ortho-tolidine in a 100 ml. Nessler tube and the volume made up with the same water.

### 3. Permanent Chlorine Standards

It cannot be emphasized too strongly that precision in the preparation of the buffer solutions hereinafter specified is necessary; therefore the following directions must be followed explicitly.

3.1. 0.5 M phosphate buffer solution. Allow a sufficient quantity of disodium hydrogen orthophosphate dodecahydrate ( $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ ) to dry over a saturated solution of potassium carbonate ( $\text{K}_2\text{CO}_3 \cdot 2\text{H}_2\text{O}$ ) until the weight remains constant for one week and weigh as  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ . If anhydrous disodium hydrogen orthophosphate ( $\text{Na}_2\text{HPO}_4$ ) is used, dry at  $110^\circ \text{C}$ . and cool in a desiccator.

Dissolve 28.66 g. of disodium hydrogen orthophosphate dihydrate ( $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ ) (or 22.86 g.  $\text{Na}_2\text{HPO}_4$ ) and 46.14 g. of anhydrous potassium dihydrogen orthophosphate ( $\text{KH}_2\text{PO}_4$ ) in 1 liter distilled water. Allow to stand for at least several days before using, to allow time for formation of

\* Of those available at the present time Zonite has been found satisfactory. Zonite contains approximately 1 per cent active chlorine.

any precipitate which must be filtered out prior to use.

3.2. 0.1 M phosphate buffer solutions. This is a standard buffer, pH = 6.45. Filter solution as prepared in A, 3.1 and dilute 200 ml. to 1 liter with distilled water.

3.3. Strong chromate-dichromate solution. Dissolve 1.55 g. of potassium dichromate ( $K_2Cr_2O_7$ ) and 4.65 g. of potassium chromate ( $K_2CrO_4$ ) in 0.1 M phosphate buffer (A, 3.2) and dilute to 1 liter with the 0.1 M phosphate buffer. This solution corresponds to the color produced by 10 ppm. of  $Cl_2$  by the standard ortho-tolidine procedure when viewed through a depth of 240–300 mm.

3.4. Dilute chromate-dichromate solution. Dissolve 0.155 g. of potassium dichromate ( $K_2Cr_2O_7$ ) and 0.465 g. of potassium chromate ( $K_2CrO_4$ ) in 0.1 M phosphate buffer (A, 3.2) and dilute to 1 liter with the 0.1 M phosphate buffer. This solution may also be prepared by diluting 100 ml. strong chromate-dichromate solution

TABLE 12.—CHLORINE STANDARDS—  
MODIFIED SCOTT FORMULA—  
0.01–1.0 PPM.\*

Chlorine	Chromate-dichromate soln.†	Chlorine	Chromate-dichromate soln.†
ppm.	ml.	ppm.	ml.
0.01	1	0.35	35
0.02	2	0.40	40
0.05	5	0.45	45
0.07	7	0.50	50
0.10	10	0.60	60
0.15	15	0.70	70
0.20	20	0.80	80
0.25	25	0.90	90
0.30	30	1.00	100

\* These standards are very close visual matches of the chlorine-ortho-tolidine color and are preferable to temporary standards which are difficult to prepare accurately.

† See A, 3.4.

TABLE 13.—CHLORINE STANDARDS—  
MODIFIED SCOTT FORMULA—  
1.0–10.0 PPM.\*

Chlorine	Chromate-dichromate soln.† at cell depth of:			
	25–50 mm.	100 mm.	200 mm.	240–300 mm.
ppm.	ml.	ml.	ml.	ml.
1	10.0	10.0	10.0	10.0
1.5	15.0	15.0	15.0	15.0
2	19.5	19.5	19.7	20.0
3	27.0	27.5	29.0	30.0
4	34.5	35.0	39.0	40.0
5	42.0	43.0	48.0	50.0
6	49.0	51.0	58.0	60.0
7	56.5	59.0	68.0	70.0
8	64.0	67.0	77.5	80.0
9	72.0	75.5	87.0	90.0
10	80.0	84.0	97.0	100.0

\* These standards are very close visual matches of the chlorine-ortho-tolidine color and are preferable to temporary standards which are difficult to prepare accurately.

† See A, 3.3.

(A, 3.3) to 1 liter with 0.1 M phosphate buffer. This solution corresponds to the color produced by 1 ppm.  $Cl_2$  by the standard ortho-tolidine procedure when viewed through all cell depths.

3.5. Modified Scott permanent chlorine standards (0.01–1.0 ppm.). The volumes of dilute chromate-dichromate solution (A, 3.4) indicated in Table 12 are pipetted into 100 ml. tubes of any uniform length and diameter or into 100 ml. volumetric flasks for use in bottles. The volume is then made up to the 100 ml. mark with 0.1 M phosphate buffer solution (A, 3.2). These standards can be read at any cell depth up to 300 mm.

3.6. Modified Scott permanent chlorine standards (1.0–10.0 ppm.). The volumes of strong chromate-dichromate solution (A, 3.3) indicated in Table 13 for the range of



cell depths given are pipetted into 100 ml. tubes of any uniform length and diameter or into 100 ml. volumetric flasks for use in bottles. The volume is then made up to the 100 ml. mark with a 0.1 M phosphate buffer solution (A, 3.2). These standards can be read for the cell depths given. Standards for cell depths other than those given can be prepared by interpolating between the depths stated in Table 13. Standards for residuals other than those given may also be prepared by interpolation of quantities at the given cell depth.

3.7. Comparison tube specifications. Variations in the viewing depth in any set of color comparison tubes, cells or bottles used in this determination shall be not more than  $\pm 3$  per cent.

3.8. Protection of standards. The tubes should be protected from dust and evaporation by sealing on micro-cover glasses with collodion, Canada balsam, or similar material. The sealing may be applied to the top of the Nessler tube by means of a camel-hair brush and the cover glass put into position promptly with forceps. After it is spot sealed, the circumference may be reinforced with additional, brush-applied, sealing material until the joining of the tube and cover glass is complete. The use of rubber stoppers is not recommended. The standards should neither be stored nor used in direct sunlight. They should be renewed whenever turbidity appears.

3.9. Commercial standards. Commercially prepared permanent standards may be used for routine tests. While experience has shown that they usually give uniform results and are

preferred in water works laboratories where facilities are not available for preparation of accurate standard solutions, they cannot be regarded as replacing the latter for research or other special purposes unless they have been checked. (Part I, Sec. 2, F, page 5.)

#### 4. Reagents

4.1. Ortho-tolidine reagent. Dissolve 1.35 g. of ortho-tolidine dihydrochloride in 500 ml. distilled water. Add this solution, with constant stirring, to 500 ml. of dil. HCl made by mixing 350 ml. distilled water and 150 ml. of concd. HCl (sp. gr. 1.18-1.19).

No directions are given for employing ortho-tolidine in the preparation of this reagent, because its use is not recommended.

4.2. Storage of ortho-tolidine reagent. The ortho-tolidine solution should be: (a) stored in amber bottles or in the dark, (b) protected at all times from direct sunlight, (c) used no longer than six months, (d) kept from contact with rubber, and (e) maintained at normal temperatures.

At temperatures less than 0° C., the ortho-tolidine will precipitate from solution and cannot be redissolved easily. The use of the reagent from which part of the ortho-tolidine has precipitated may lead to errors due to a deficiency of ortho-tolidine.

#### 5. Procedure

5.1. Addition of sample to reagent. Use 0.5 ml. ortho-tolidine reagent in 10 ml. cells, 0.75 ml. in 15 ml. cells, 5 ml. in 100 ml. tubes and

the same ratio for other volumes. Place ortho-tolidine reagent in the Nessler tube, colorimeter cell, or other container, add sample to the proper mark or volume and mix.

5.2. Temperature. When the temperature of the sample is less than 20° C., bring the treated sample to that temperature quickly after mixing the sample with ortho-tolidine. If a comparator cell is used, place it in hot water until the specified temperature is reached.

If a Nessler tube is used, it may be handled in the same manner as the cell, or the contents may be transferred to a flask for heating. For approximate results in the field, other means of heating may be employed, but the results will not be comparable.

5.3. Color development and comparison. Place the treated sample (A, 5.1) in the dark during color development. Maximum color develops in less than 5 minutes and fading occurs thereafter; therefore, to obtain the proper color value, the reading should be made within 5 minutes.

When all chlorine in the sample is in the free condition maximum color appears almost instantaneously.

Color comparison should be made against standards which occupy the same depth of tube or cell as the sample.

5.4. Compensation for interference. The presence of natural color or turbidity in the sample interferes with the reading of the color developed. When viewing sample and standard horizontally, this interference may be compensated for by placing an untreated sample of the same thickness behind the standard

and clear water behind the sample being compared.

## B. IODOMETRIC METHOD

The iodometric method for the determination of residual chlorine in water may be made more precise than the ortho-tolidine method, particularly when residual chlorine is greater than 1 ppm. When the titration is made without acid, the interfering effect of nitrites and manganic and ferric salts is minimized. Generally, the starch-iodide method carried out in acid solution yields higher values than the neutral starch-iodide titration. The acid titration therefore is preferred when interfering substances are known to be absent. Acetic acid should be used. Sulfuric acid may be used only when the analyst has satisfied himself that interfering substances are absent, but *hydrochloric acid should never be used.*

### 1. Standardization of Chlorine Solution

1.1. Volume of sample. In standardizing the chlorine solution (A, 2.2) for temporary standards, the volume taken for titration should be such as to require not more than 20 ml. 0.01 N sodium thiosulfate.

In standardizing chlorine water solution (Part I, Sec. 50, A, 1.2, page 103) for determining chlorine demand, the volume taken for titration should be such as to require not more than 20 ml. of 0.1 N sodium thiosulfate.

1.2. Preparation for titration. Proceed as in B, 3.2. Distilled water may be added at the discretion of the analyst if a larger volume is preferred for titration.

1.3. Titration. Titrate the chlorine solution as in B, 3.3.

1.4. Calculation of results. In standardizing chlorine solution for temporary standards:

mg. chlorine per ml. =  $[\text{ml. } 0.01 \text{ N Na}_2\text{S}_2\text{O}_3 \times 0.3546] \div \text{ml. sample titrated.}$

In standardizing chlorine water solution for determining chlorine demand:

mg. chlorine per ml. =  $[\text{ml. } 0.1 \text{ N Na}_2\text{S}_2\text{O}_3 \times 3.546] \div \text{ml. sample titrated.}$

## 2. Reagents

2.1. Potassium iodide solution. Dissolve 75 g. cp. KI (free from iodine and iodate) in 1 liter freshly boiled and cooled distilled water. If difficulty is experienced with stability of the solution, the solid substance may be used directly, since it is very soluble.

2.2. 0.1 N sodium thiosulfate. Make 0.1 N sodium thiosulfate by dissolving at least 25 g. of cp.  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  in 1 liter of freshly boiled distilled water, standardizing it against potassium dichromate (2.3 below) after at least two weeks' storage. Boiled water is used because bacterial action decomposes 0.1 N thiosulfate. Trouble may be avoided by the addition of a few ml. of chloroform.

2.3. Standardization of 0.1 N sodium thiosulfate. To 80 ml. of distilled water add, with constant stirring, 1 ml. of concd.  $\text{H}_2\text{SO}_4$ , 10 ml. of 0.1 N  $\text{K}_2\text{Cr}_2\text{O}_7$  soln. and 10 ml. of KI soln. (2.1).

Allow to stand six minutes in subdued light at laboratory temperature,

and titrate with the thiosulfate being standardized.

2.4. 0.01 N sodium thiosulfate. Stability of 0.01 N thiosulfate is improved if it is prepared by diluting an aged 0.1 N solution (made as directed in B, 2.2) with freshly boiled distilled water. Boiled water is used because bacterial action decomposes 0.01 N thiosulfate. Trouble may be avoided by the addition of a few ml. of chloroform. For accurate work, this solution should be standardized daily, in accordance with the directions given in 2.3 above, using 0.01 N  $\text{K}_2\text{Cr}_2\text{O}_7$  soln., if desired. The use of an automatic burette, of a type in which rubber does not come in contact with the solution is advisable.

2.5. Starch solution. To 5 g. starch (potato, arrowroot or soluble) in a mortar, add a little cold water and grind to a thin paste. Pour into 1 liter of boiling distilled water, stir and allow to settle overnight. Use the clear supernatant. The solution should be preserved with salicylic acid (1.25 g. per liter), or with zinc chloride (4 g. per liter).

2.6. Acid solution. Use either (a) a solution of acetic acid, made by diluting 500 ml. glacial acetic acid to 1 liter with distilled water, or (b) a solution of sulfuric acid, made by adding 20 ml. of concd.  $\text{H}_2\text{SO}_4$  (sp. gr. 1.84) to approximately 750 ml. distilled water and diluting to 1 liter. Add the acid to the water slowly, with constant stirring.

## 3. Procedure

3.1. Volume of sample. The amount of sample to be taken for titration is governed by the concentra-



tion of chlorine in the sample. It is suggested that, for quantities of residual chlorine of 1.0 ppm. or less, 1 liter be titrated; for residual chlorine concentrations between 1 ppm. and 10 ppm., 500 ml.; for residual chlorine concentrations above 10 ppm., proportionately less of the sample should be used. It is preferable to use sufficient sample so that not more than 20 ml. of 0.01 N thiosulfate is required.

3.2. Preparation for titration. If no interfering substances are present, place 10 ml. acid, or sufficient to reduce the pH to between 3.0 and 4.0, in a flask or white porcelain casserole, add 10 ml. of KI soln., pour in the sample and mix with a stirring rod. Omit the acid if interfering substances are present.

3.3. Titration. Add 0.01 N thiosulfate from a burette until the yellow color of the liberated iodine is almost discharged. When liter samples are titrated, add 5 ml. starch, reducing this amount with smaller samples. The amount of starch used should be sufficient to give a deep blue color. After the addition of starch, titrate carefully, but rapidly, to the end point. Long contact of iodine and starch produces a blue compound which is decolorized with difficulty.

3.4. Calculation of results.

$$\text{ppm. Cl}_2 = \text{ml. 0.01 N Na}_2\text{S}_2\text{O}_3 \times 0.3546 \times 1000 \div \text{ml. sample.}$$

#### C. ORTHO-TOLIDINE FLASH TEST METHOD

Chlorine in water may be present in the form of free available chlorine (as HOCl and/or OCl<sup>-</sup> ions) or com-

bined available chlorine (chloramines and/or other chloro-nitrogenous compounds). Both of these forms of chlorine may exist at the same time. A qualitative determination of the presence of free chlorine is afforded by the Laux "flash test." Oxidized manganese will produce flash colors with ortho-tolidine reagent in this test, similar in shade and speed of development to free available chlorine residuals.

#### 1. Procedure

Flash test. Place a sample of the water under test in a comparator cell or other small container, of such dimensions as to insure immediate and complete mixture of the reagent with the sample. Hold the container against a white background and, using a medicine dropper, add with force from 0.5 to 1 ml. of ortho-tolidine reagent to the sample of water, paying close attention to color development.

If color development is instantaneous, free available chlorine is present, unless the sample contains oxidized manganese. With practice, as little free available chlorine as 0.1 ppm. may be detected in this manner.

Delay in color development indicates the presence of combined available chlorine and not free available chlorine.

#### D. ORTHO-TOLIDINE-ARSENITE (OTA) METHOD

Within the limitations specified, the ortho-tolidine-arsenite (OTA) test permits the relative amounts of free available chlorine, combined available chlorine, and color due to interfering substances in water to be measured.

The limitations of this test are: in samples containing a high proportion of combined available chlorine, it may indicate more free available chlorine than is actually present; in samples containing a low proportion of combined available chlorine, it may indicate less free available chlorine than is actually present.

Precision of results depends on strict adherence to conditions of the test. The conditions are: (a) the time intervals between addition of reagents; (b) the relative concentration of free available chlorine and of combined available chlorine in the sample; (c) the temperature of the sample.

The temperature of the sample under examination should never be above 20° C., and the precision of the test increases with decreasing temperature.

### 1. Apparatus

1.1. Color and turbidity compensating residual chlorine comparator. As an alternate, use three French square bottles of 1 oz. or 2 oz. capacity and permanent chlorine standards (prepared as specified in A, 3 or commercially prepared) in similar bottles.

### 2. Reagents

2.1. Ortho-tolidine reagent. The same reagent as that in A, 4.1.

2.2. Arsenite reagent. Dissolve 5 g. of sodium arsenite (sodium meta arsenite),  $\text{NaAsO}_2$ , in distilled water and dilute to 1 liter.

### 3. Procedure

Label three comparator cells, or French square bottles, A, B, and OT.

Use 0.5 ml. of ortho-tolidine reagent in 10 ml. cells, 0.75 ml. in 15 ml. cells, and the same ratio for other volumes of sample. Use the same volume of arsenite reagent as is specified for ortho-tolidine reagent.

3.1. To cell A, containing ortho-tolidine reagent, add a measured volume of water sample. Mix quickly and immediately (within 5 seconds) add arsenite reagent. Mix quickly and compare with color standards as rapidly as possible. Record the result. The value obtained (A) represents free available chlorine and interfering colors.

3.2. To cell B, containing arsenite reagent, add a measured volume of water sample. Mix quickly and immediately add ortho-tolidine reagent. Mix quickly and compare with color standards as rapidly as possible. Record the result as the B-1 value.

Compare with color standards again in exactly 5 minutes and record the result as the B-2 value. The values obtained represent the interfering colors present in the immediate reading (B-1) and in the 5 minute reading (B-2).

3.3 To cell OT, containing ortho-tolidine reagent, add a measured volume of water sample. Mix quickly and compare with color standards in exactly 5 minutes. Record the result. The value obtained (OT) represents the total amount of residual chlorine present and the total amount of interfering colors.

3.4. Determination of results.

*Total Residual Chlorine.* From the value of OT subtract the value of B-2. The difference equals total residual chlorine.

*Free Available Chlorine.* From the value of *A* subtract the value of *B-1*. The difference equals free available chlorine.

*Combined Available Chlorine.* From the value of total residual chlorine subtract the value of free available chlorine. The difference equals combined available chlorine.

*Interfering Colors.* The *B-1* value represents the color produced by interfering substances immediately and the *B-2* reading represents the color produced by interfering substances in 5 minutes.

#### E. DROP DILUTION METHOD FOR FIELD USE

This test is designed for field measurements of residual chlorine, where concentrations are greater than 10 ppm. and where speed of estimation is of importance. It is particularly useful in connection with the sterilization of mains or tanks, where laboratory apparatus is ordinarily lacking. The test is not intended to displace titration methods and should not be used where extreme accuracy, such as standardization of solutions, is desired.

##### 1. Apparatus

The determination may be made in comparators or in Nessler tubes, with due consideration given to the amount of chlorine solution taken for test.

The medicine dropper used in any determination by this method should be checked to see that it delivers approximately 20 drops per ml.

##### 2. Reagents

2.1. Ortho-tolidine reagent. The ortho-tolidine reagent is the same as that in A, 4.1.

##### 3. Procedure

3.1. Reagent and sample. If a comparator, in which the cell contains 10 or 15 ml., is used, place 0.5 ml. ortho-tolidine solution in the cell. If a 100 ml. Nessler tube is used, place 5.0 ml. ortho-tolidine solution in the tube.

Fill the cell or tube to the mark with distilled water and mix thoroughly.

Add to the cell or tube one drop of water under test. Mix thoroughly and read as provided in A, 5.3, page 98.

If the addition of one drop of water under test produces no color, discard the contents of the cell and refill with ortho-tolidine and distilled water as before and add two drops of the water under test. Continue this procedure with increasing amounts of sample until an easily readable color (not less than 0.1 ppm.) is produced.

3.2. Contact time. A sample of water with such concentrations of chlorine as may be determined by this method contains only free available chlorine and traces of or no combined available chlorine. For this reason the color development is very rapid and the reading may be made almost at once.

3.3. Calculation. Calculation of results may be made by using the following formula:

$$\left[ \text{volume of cell, ml.} \div \text{ml. of sample} \right] \times \text{reading} = \text{ppm. residual chlorine.}$$

##### EXAMPLE:

$$\left[ 15 \text{ (capac. of cell)} \div 0.05 \text{ (1 drop)} \right] \times 0.10 \text{ (reading)} = 30 \text{ ppm.}$$



## BIBLIOGRAPHY

- ELLMs, J. W., AND HAUSER, S. J. Ortho-tolidine as a reagent for the colorimetric estimation of small quantities of free chlorine. *Ind. Eng. Chem.*, 5, 915, 1030 (1913).
- RACE, J. *Chlorination of Water*. J. Wiley & Sons, New York (1918).
- THERIAULT, E. J. The ortho-tolidine reagent for free chlorine in water. *Pub. Health Repts.*, 42, 668 (1927).
- ADAMS, HOWARD W., AND BUSWELL, A. M. Ortho-tolidine test for chlorine. *J. Amer. W. W. Assn.*, 25, 1118 (1933).
- SCOTT, R. D. Eliminating false chlorine tests. *J. Amer. W. W. Assn.*, 26, 634 (1934).
- DAVIS, W. S., AND KELLY, C. B. Photo-discoloration of ortho-tolidine and artificial standards for the chlorine test in water. *J. Amer. W. W. Assn.*, 26, 759 (1934).
- SCOTT, R. D. Effect of iron in the determination of residual chlorine. *J. Amer. W. W. Assn.*, 26, 1234 (1934).
- HULBERT, ROBERTS. Chlorine and the ortho-tolidine test in the presence of nitrite. *J. Amer. W. W. Assn.*, 26, 1638 (1934).
- SCOTT, R. D. Improved standards for the residual chlorine test. *Water Wks. & Sew.*, 82, 399 (1935).
- GRIFFIN, A. E. Evaluation of residual chlorine. *J. Amer. W. W. Assn.*, 27, 889 (1935).
- HALLINAN, F. J., AND THOMPSON, W. R. A critical study of the thiosulfate titration of chlorine. *J. Am. Chem. Soc.*, 61, 265 (1939).
- LAUX, P. C. Break-point chlorination at Anderson. *J. Amer. W. W. Assn.*, 32, 1027 (1940).
- GRIFFIN, A. E., AND CHAMBERLIN, N. S. Estimation of high chlorine residuals. *J. Amer. W. W. Assn.*, 35, 571 (1943).
- CHAMBERLIN, N. S., AND GLASS, J. R. Colorimetric determination of chlorine residuals up to 10 ppm. with ortho-tolidine. *J. Amer. W. W. Assn.*, 35, 1065, 1205 (1943).
- AMER. W. W. ASSN. COMM. Report on control of chlorination. *J. Amer. W. W. Assn.*, 35, 1315 (1943).
- GILCREAS, F. W., AND HALLINAN, F. J. The practical use of the ortho-tolidine-arsenite test for residual chlorine. *J. Amer. W. W. Assn.*, 36, 1343 (1944).

## 50. Chlorine Demand

## A. METHOD FOR LABORATORY USE

The chlorine demand method presented is designed to determine the so-called immediate demand or any other demand using longer contact time. Chlorine water is used as the source of chlorine when liquid chlorine is used in operation.

Commercial solutions of hypochlorite (Part I, Sec. 49, A, 2.2, page 95) are more convenient for use in the field or when facilities are not available for standardizing chlorine water. In general, the results will be of the same order of accuracy as with chlorine water.

The chlorine demand of water is the difference between the amount of chlorine applied and the amount of residual chlorine remaining at the end of the contact period. The demand for any given water varies with the amount of chlorine applied, time of contact and temperature. For comparative purposes it is imperative that all conditions be stated. The smallest amount of residual chlorine considered at all significant is 0.1 ppm. Treatment of water beyond this point usually results in a series of increasing demands.

## 1. Reagents

1.1. Ortho-tolidine reagent (Part I, Sec. 49, A, 4.1, page 97) or iodimetric reagents (Part I, Sec. 49, B, 2.1-2.4, page 99).

1.2. Chlorine water solution. A suitable solution may be obtained from the chlorinator solution hose or by bubbling chlorine gas through water obtained from the laboratory tap. Due to instability of chlorine

water, it is necessary to make up fresh solutions daily. Under any conditions standardize the chlorine solution immediately prior to the start of each test. The use of amber bottles is recommended. When hypochlorite is used in plant operation, it may be used for this test.

The strength of the chlorine water or hypochlorite solution should be such that the volume of the treated sample will not be increased by more than 5 per cent.

Standardize the chlorine solution iodimetrically (Part I, Sec. 49, B, 1, page 98).

## 2. Procedure

2.1. Volume of samples. Measure at least ten equal portions of the water under test. Use beakers or other glass containers of ample capacity to permit mixing the chlorine with the water.

If the object of the test is to determine chlorine demand, the volume of the sample in each container may be no more than 200 ml. If the purpose of the test is to relate chlorine demand to bacterial removal, the effect on taste and odor or the chemical constituents of the water, samples of 500 ml. or more will be required.

2.2. Addition of chlorine water. The amount of chlorine added to the first jar should be such that no chlorine remains at the end of the contact period, especially when low demands are being studied. Add increasing amounts of chlorine to successive portions in the series. The increase between portions may be as low as 0.1 ppm. for determining low

demands and up to 1.0 ppm. or more for exploring higher demands.

Mixing is imperative while the chlorine solution is being added to the sample.

2.3. Contact time. To determine in advance the effect of chlorination, the plant contact time and temperature should be duplicated in the laboratory. For plant control, other and shorter contact periods at some controlled temperature generally can be found. During the contact period, the chlorinated samples must be protected from strong daylight.

2.4. Examination of samples. At the end of the contact period, remove an equal amount from each portion and determine the residual chlorine by a method described in Part I, Sec. 49, page 92.

It will aid in studying the results to plot the residual chlorine or the amount consumed versus the dosage.

Samples for bacteriological examination may be removed at desired intervals.

Taste and odor of the treated samples may be observed at ordinary temperatures without de-chlorination. For odor observation at elevated temperatures, samples should be de-chlorinated before heating. Choice of the de-chlorinating agent must be made with due regard to its effect on odor in the water under examination. Generally, sodium bisulfite, using only small excess, is satisfactory.

## B. METHOD FOR FIELD USE

This test is designed for the measurement of the chlorine demand in the plant or the field when adequate facilities or personnel are not avail-

able to employ the more exact method (A). Results obtained in this test are approximations only.

### 1. Apparatus

1.1. One medicine dropper which will deliver 20 drops per ml.

1.2. Ten 1 qt. fruit jars marked at the 500 ml. point.

1.3. Ten 2 oz. bottles marked at the 20 ml. point.

1.4. Glass stirring rod.

1.5. Glass-stemmed thermometer.

1.6. Permanent chlorine standards (Part I, Sec. 49, A, 3, page 95) or commercial standards.

### 2. Reagents

2.1. Ortho-tolidine reagent (Part I, Sec. 49, A, 4.1, page 97).

2.2. Chlorine solution. A commercial preparation containing approximately 1 per cent available chlorine by weight may be used.\* For this test its volumetric measurement is sufficiently accurate.

### 3. Procedure

3.1. Measurement of samples. Fill each jar to the 500 ml. mark with the water under test. Record temperature at which samples are held.

3.2. Addition of chlorine. While stirring constantly, add 1 drop of chlorine solution (2.2) to water in the first fruit jar, 2 drops to that in the second jar, 3 drops to that in the third jar, etc. Each drop of chlorine solution corresponds to a chlorine dose of approximately 1.0 ppm.

3.3. Contact time. The contact time is a variable factor depending entirely on the results desired. Gen-

erally, the purpose of a chlorine demand test is to determine the amount of chlorine required to produce a definite residual after a definite time interval. It is recommended, therefore, that the testing be carried out at the end of the contact period corresponding to the contact time at the point of control.

This may vary from a few minutes to several hours. Under some circumstances it is desirable to make several chlorine determinations at different times of contact, such as 15, 30, 60 minutes, etc. Such a procedure will give an indication of the stability of the chlorine as related to time, which will be very useful information in plant control.

The time of contact must be recorded. During the contact period the chlorinated samples must be protected from strong daylight.

3.4. Examination of samples. At the end of the contact period, remove a 20 ml. portion from each sample, place it in a 2 oz. bottle and add 1 ml. ortho-tolidine to each 20 ml. Determine residual chlorine by comparison with permanent standards.

3.5. Calculation.  $\text{ppm. Cl}_2 \text{ demand} = \text{ppm. Cl}_2 \text{ added} - \text{ppm. residual Cl}_2$ . This chlorine demand refers to the sample at the respective dosage, contact time, and temperature. Plotting the residual chlorine or the amount consumed versus the chlorine added will aid in studying the results.

### BIBLIOGRAPHY

A. W. W. A. COMMITTEE. Report on control of chlorination. *J. Amer. W. W. Assn.*, 35, 1315 (1943).

\* Of those available at the present time, Zonite has been found satisfactory.



## 51. Dissolved Oxygen

In alkaline solution, oxygen oxidizes manganous ion to manganic ion. Manganic ion in turn oxidizes iodide ion to free iodine in amount equivalent to the oxygen originally present. After acidifying the solution, the free iodine is determined by titration with sodium thiosulfate. The end point is detected either by using starch as an internal indicator or by using electrometric equipment.

It is obvious that oxidizing and reducing impurities present may affect the results obtained. For extremely low concentrations of dissolved oxygen, the amount of oxygen present in the reagent solutions will be of significance. *The following procedures are primarily for use on boiler waters.*

For the determination of dissolved oxygen in natural waters and for a thorough discussion of interfering substances, see Part II, Sec. 10, page 124.

### 1. Sampling

Collect a representative sample in a vessel which will not subject the sample to atmospheric contamination and which will also permit the reagent solution to be added without atmospheric contamination.

Secure the sample as close to 21° C. as possible, passing it through a cooling coil if necessary. Joints should be brazed wherever possible. Cocks and unions may be made tight by shellacing; red lead used for this purpose introduces large errors, and

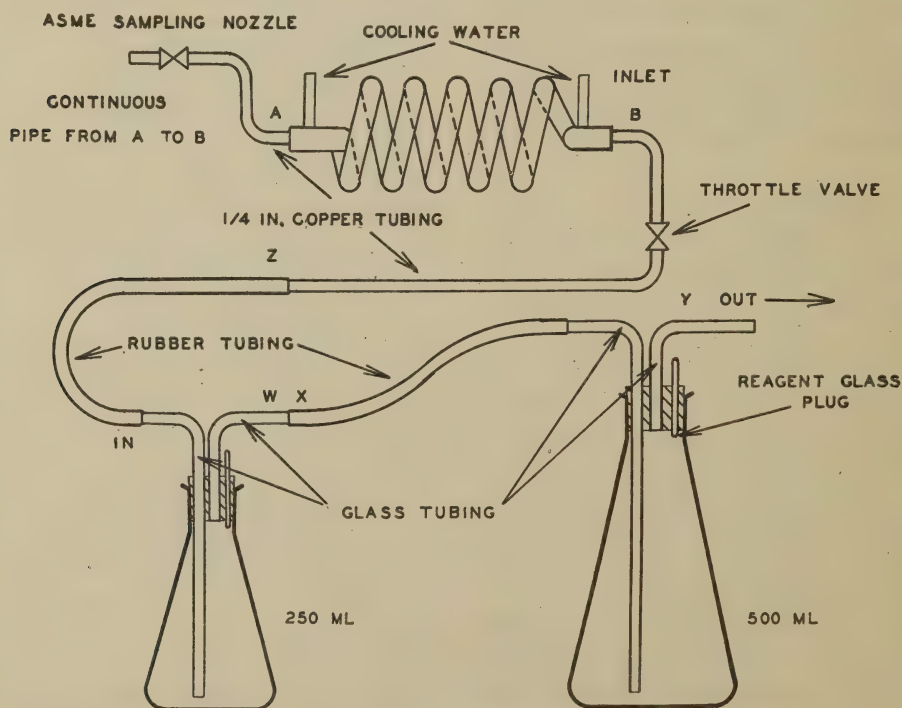


FIG. 7. DISSOLVED OXYGEN IN BOILER WATER—SAMPLING FLASK.

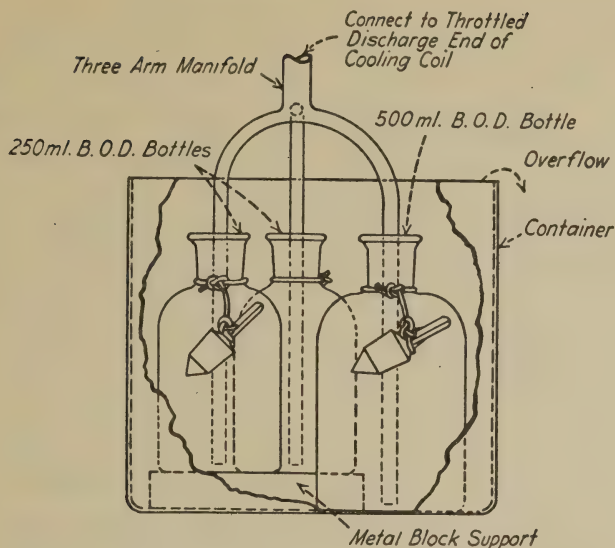


FIG. 8. NEW SAMPLING ASSEMBLY FOR SCHWARTZ AND GURNEY OXYGEN TEST.

lines in which it has been used should be flushed out one or more days before a sample is drawn.

New rubber tubing should not be used at any point, since the sulfur which it introduces into the sample will cause appreciable errors. Sulfur may be removed by boiling the tubing six hours in 20 per cent caustic soda solution and then flushing out thoroughly for several hours. The sampling line should be thoroughly flushed for approximately 30 minutes. A flow of 400 ml. per minute is recommended. Allow the water to flow through the sample vessel for 15 minutes.

The sampling vessels may be Erlenmeyer flasks with three hole rubber stoppers, modified gas sampling tubes or B.O.D. bottles (Part II, Sec. 11, page 140) placed inside a bucket.

1.1. The following sampling procedure is recommended for use with

Erlenmeyer flasks as shown in Fig. 7. Connect to Z and allow the 250 ml. flask to overflow. The stopper should not be inserted until the flask is well flushed out. Insert the stopper firmly, and then the reagent plug. No bubbles should be visible at any point. Connect the end X of the rubber tube from the 500 ml. flask to W on the 250 ml. flask and repeat the operation for the 500 ml. flask. Allow the water to run for 15 minutes. To disconnect the samples from the line pinch the rubber tube at X and quickly loop over and connect to Y. Likewise pinch at Z and loop over to W. The samples are now ready to be fixed.

1.2. The following sampling procedure is recommended for use with B.O.D. bottles as shown in Fig. 8. A manifold is attached to the sampling line, the arms of which extend into the three separate B.O.D. bottles placed in a container so that the over-

flow will leave the tops of the bottles under at least one inch of water. Each bottle has its stopper wired on. The two 250 ml. bottles rest upon a support so that the necks of all three bottles are on the same level.

With the water supply of the cooling coil adjusted to give hot sample without flashing, open the throttle of the sampling line enough to flow a steady but not turbulent stream of hot water into the bottles at a rate of about 1 liter per minute, thus filling the 500 ml. bottle from the manifold in about  $1\frac{1}{2}$  minutes.

Allow the container to overflow about three times its volume and then turn on the cooling water fully in the jacket, leaving the rate of flow of sam-

ple unchanged. Allow the container to overflow about five times its volume and slowly raise the manifold until all arms disengage from the bottles. Lower the manifold into the container with the arms outside the bottles.

Stopper the bottles and examine each one for entrained air bubbles. (If any are found, disengage them and flow more sample through all the bottles using about five times the volume of the container. One visible air bubble can ruin a sample.) Keep the water to be sampled flowing through the manifold and into the container. The samples are now ready to be fixed, as in 4.1.2.

1.3. The following procedure is

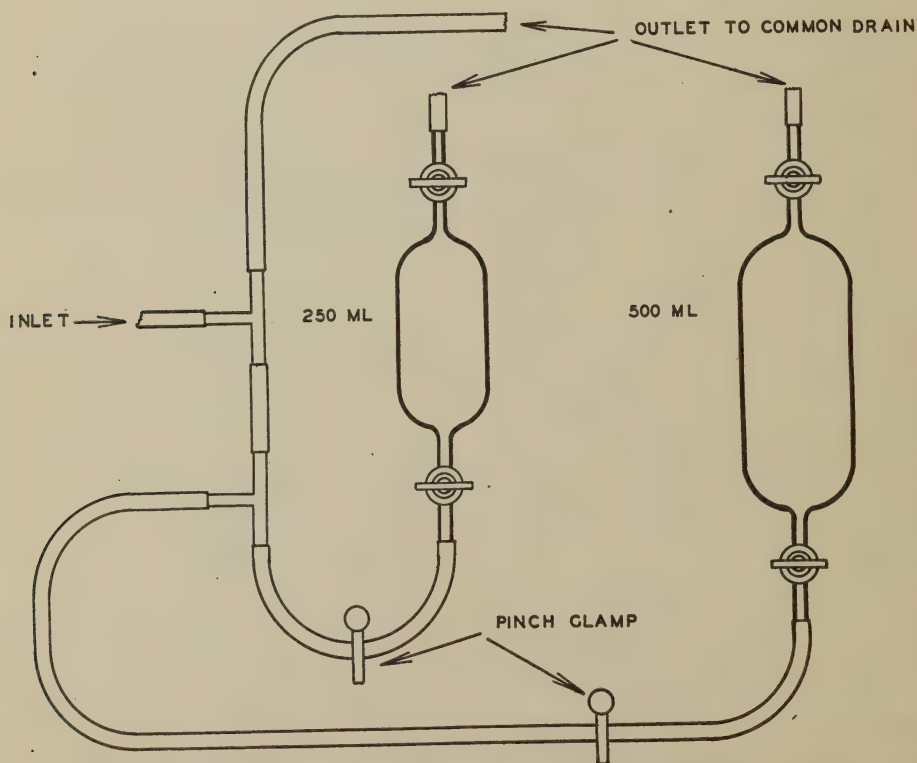


FIG. 9. MODIFIED GAS SAMPLING TUBES FOR DISSOLVED OXYGEN SAMPLING.



recommended for use with modified gas sampling tubes as shown in Fig. 9. Connect the tubes to the sample line using a Y or T outlet. Maintain equal flow to both tubes. Allow the water to flow through both tubes for 15 minutes. Collect 250 ml. of water in an open flask or bottle. The stems of the tubes should be marked so that 2 ml. of reagents can be added from either end without using all the solution in the stem.

## 2. Apparatus

2.1. Electrometric titration equipment.

2.2. Sampling vessel, 500 ml.—1.

2.3. Sampling vessel, 250 ml.—2.

## 3. Reagents

3.1. Manganous sulfate solution. Dissolve 480 g. of  $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$  or 400 g. of  $\text{MnSO}_4 \cdot 2\text{H}_2\text{O}$  in distilled water; filter and dilute to 1 liter. When uncertainty exists regarding the water of crystallization, a solution of equivalent strength may be obtained by adjusting the specific gravity of the solution to a value of 1.270 at 20° C. The  $\text{MnSO}_4$  solution should not liberate more than traces of iodine when added to an acidified solution of potassium iodide.

3.2. Alkaline potassium iodide solution. Dissolve 700 g. of KOH in 700 ml. of distilled water and cool to room temperature. Dissolve 150 g. of KI (iodate free) in 200 ml. of distilled water and cool to room temperature. Mix the two solutions and dilute, with distilled water, to 1 liter. Potassium and sodium salts may be used interchangeably (500 g. of NaOH, 135 g. of NaI). The reagent

should be practically free from carbonates. It should not give any color with starch when diluted and acidified.

3.3. Sulfuric acid solution. Add carefully 750 ml. of concd.  $\text{H}_2\text{SO}_4$  (sp. gr. 1.83 to 1.84) to 250 ml. of distilled water and cool to room temperature. Dilute with distilled water to 1 liter.

3.4. Sodium thiosulfate stock solution, 0.1 N. Dissolve 24.84 g. of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  in distilled water and dilute to 1 liter. Standardize against potassium bi-iodate or potassium iodate. The solution may be preserved by (1) chloroform, 5 ml. per liter (after making up to the mark), or (2) the addition of 0.4 g. of NaOH per liter.

3.5. Sodium thiosulfate standard solution, 0.01 N. Dilute 10 ml. of the 0.1 sodium thiosulfate solution (3.4) to 1 liter. Prepare fresh.

3.6. Starch solution, approximately 0.5 per cent. Grind 5 or 6 g. of potato starch in a mortar with a small quantity of water. Pour into 1 liter of boiling water, stir and allow to settle overnight. Use the clear supernatant. The solution may be preserved with 1.25 of salicylic acid per liter.

3.7. Potassium bi-iodate standard solution, 0.01 N. Dissolve 0.3250 g.  $\text{KH}(\text{IO}_3)_2$  in distilled water and dilute to 1 liter.

## 4. Procedure

4.1. Fixing the samples.

4.1.1. Erlenmeyer flasks.

Both samples are treated as follows: Remove the reagent plug and carefully introduce 2 ml. of  $\text{MnSO}_4$  soln.

The solution should start flowing just before it enters the water in the reagent plug hole and should be kept flowing until it is removed. Replace the reagent plug carefully, pinch the rubber tube loop against the side of the flask and whirl the flask in a horizontal orbit.

Repeat this procedure introducing 2 ml. of alkaline iodide solution using a second pipette. In the same manner 2 ml. of  $\text{H}_2\text{SO}_4$  soln. are added using a third pipette. Pinch the rubber tubing tightly, remove the reagent plug and the rubber stopper carrying the inlet and exit tubes. The contents of the tubes are washed to waste.

4.1.2. B.O.D. bottles. Add reagents in same manner as in 4.1.1.

4.1.3. Modified gas sampling tubes. Introduce the reagents from alternate ends of the sampling tube, making sure that the stem is thoroughly washed out each time. Mix after each reagent addition. In each instance the lower stopcock is opened slowly after the upper stopcock has been first opened.

4.2. Oxygen concentration above 0.5 ppm.

4.2.1. Collect a suitable size sample. Introduce 2 ml. of  $\text{MnSO}_4$  soln. and mix. Add 2 ml. of alkaline KI and mix. Add 2 ml. of  $\text{H}_2\text{SO}_4$  soln. and mix. Titrate the sample, or an aliquot, with 0.1 N  $\text{Na}_2\text{S}_2\text{O}_3$  adding 2 ml. of starch solution when the end point, as observed by the color of the solution, is approached.

4.2.2. Collect one 500 ml. and two 250 ml. samples simultaneously using either Erlenmeyer flasks, modified gas sampling tubes, or B.O.D. bottles placed in a bucket.

Into one of the 250 ml. samples and the 500 ml. sample, introduce the  $\text{MnSO}_4$  soln., the alkaline KI soln., and the  $\text{H}_2\text{SO}_4$  soln. in the manner specified in 4.1.

Add the 250 ml. of untreated water to the 250 ml. of treated water. Add between 0.25 and 0.50 ml. of potassium bi-iodate or iodate solution to both solutions in exact amount.

Titrate both solutions to the nearest 0.01 ml. with 0.01 N  $\text{Na}_2\text{S}_2\text{O}_3$  soln. using electrometric titration equipment preferably, but if such apparatus is unavailable, use 2 ml. of starch solution. In the latter case, a comparison sample of distilled water will prove helpful in the titration.

$$\text{ppm. oxygen} = [0.80 \times \text{ml. } 0.1 \text{ N } \text{Na}_2\text{S}_2\text{O}_3 \times 1000] \div \text{ml. sample.}$$

$$\text{ppm. oxygen} = 0.32 (\text{ml. } 0.01 \text{ N } \text{Na}_2\text{S}_2\text{O}_3 \text{ used for 500 ml. sample} - \text{ml. } 0.01 \text{ N thio-sulfate used for two 250 ml. samples}).$$

## BIBLIOGRAPHY

- SCHWARTZ, M. C., AND GURNEY, W. B. *Proc. A. S. T. M.*, **34**, *II*, 796-820 (1934).  
 SCHWARTZ, M. C. *University Studies No. 21*, 46 pp., Louisiana State University Press (1935).  
 ADAMS, R. C., BARNETT, R. E., AND KELLER, D. E., JR. *A. S. T. M., Preprint No. 90* (1943).  
 DAUGHERTY, T. H. *Proc. A. S. T. M.*, **37**, *II*, 615-33 (1937).  
 ULMER, R. C., REYNAR, J. M., AND DECKER, J. M. *Proc. A. S. T. M., Preprint No. 91* (1943).

## 52. Hydrogen Sulfide

Methods for the determination of hydrogen sulfide are described in Part II, Sec. 20, page 152.

### A. SAMPLING

Water containing dissolved sulfides readily loses hydrogen sulfide, particularly if the pH of the sample is

low. Oxygen destroys sulfide by oxidation, particularly if the pH of the sample is high. Aeration of the samples should therefore be avoided. It is advisable to fix the sample by addition of a suitable quantity of zinc acetate solution which precipitates the sulfide as zinc sulfide. In case the sample is not fixed in this manner, it should be analyzed immediately.

#### B. FREE OR UNCOMBINED HYDROGEN SULFIDE BY CALCULATION

The concentration of hydrogen sulfide may be calculated if the pH of the sample and the dissolved sulfide concentration are determined or known.

The procedure is described in Part II, Sec. 20, B, 2.3, page 153.

### 53. Methane

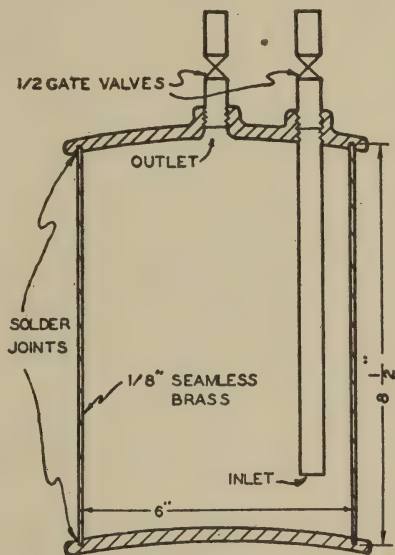
Methane gas is colorless, odorless, and tasteless. It is inflammable and explosive on ignition when mixed, in concentrations of 5 to 15 per cent, with air. Several serious explosions have occurred due to the unrecognized presence of this gas in ground water supplies. Every precaution should be taken to prevent the release of this gas from the water in unventilated chambers where it might accumulate to an explosive concentration.

In many cases qualitative recognition of the presence of this gas is sufficient without a quantitative analysis.

Special sampling and extraction method precautions are necessary when methane is separated from the water. Methane is determined by usual gasometric methods.

#### 1. Sampling

Fig. 10 shows a suitable container of approximately 1 gallon capacity. Its exact volume should be determined by calibration. Fill the container by connecting a rubber hose from a tap (or from some other pressure source of the water to be examined) to the inlet valve. Secure



CROSS SECTION OF CONTAINER

FIG. 10. SAMPLER FOR COLLECTING METHANE IN WATER.

a representative sample by passing sufficient water through the container to cause at least 2 gallons to overflow to waste. Close the valves and transport the container to the laboratory on its side to prevent possible leakage of gas through the valves.

#### 2. Procedure

The gases are boiled out of the water in the container into a gas collector as follows: Set up a collector consisting of a glass tube 1 inch in



diameter and 4 feet in length provided with one hole rubber stoppers at each end carrying glass stop-cocks inserted flush with the inner end of each stopper. This tube should be calibrated in volumes of not greater than 10 ml. The bottom stop-cock of the gas collector is next connected by a 5 foot rubber tube to a moveable reservoir (approximately 1 liter capacity). By means of this reservoir the collector is filled flush with the top of the upper stop-cock tube with a saturated solution of sodium sulfate made acid to methyl red.

Place the sampler in an upright position as shown in Fig. 10 and support it so that any convenient source of heat can be applied to the bottom. A 2 foot rubber tube is connected to the outlet valve of the sampler and filled with the saturated  $\text{Na}_2\text{SO}_4$  soln. It is then connected to the top stop-cock of the gas collector, care being taken to avoid the entrance of air bubbles.

Place the sulfate solution reservoir at a level such that the upper surface of the liquid is approximately 1 foot above the bottom of the gas collector tube (reservoir should contain not more than 400 ml. of solution at this point). Open stop-cocks and valves in the following order: first, the bottom stop-cock of the gas collector; second, the top stop-cock of the gas collector; and third, the outlet valve of the sampler.

Any convenient source of heat (Bunsen burner or electric hot plate) is applied to the bottom of the sampler and the temperature of the sample raised to boiling. Heating is continued until the level of the solution

inside the gas collector has receded to about the level of the liquid in the solution reservoir.

With fingers protected against heat, simultaneously close the stop-cock at the top of the gas collector and disconnect it from the rubber tube to the sampler and then immediately remove the heat from the bottom of the sampler. (It is convenient to set up this apparatus near a sink so that the hose connection from the sampler to the collector can be allowed to discharge into the sink.)

Allow the gases in the collector to cool to room temperature, close the bottom stop-cock and turn the collector to a horizontal position and back to vertical two or three times to insure uniform mixture of the gases.

With the collector in a vertical position open the lower stop-cock and level the liquid in the reservoir with the liquid in the collector to determine the volume, noting atmospheric temperature and pressure. Connect the upper stop-cock to a suitable gas pipette or gas burette, and transfer a known aliquot for analysis. The aliquot is analyzed for carbon dioxide and methane according to usual methods of gasometric analysis.

$$\text{ppm. CH}_4 = (\text{ml. gas extracted} \times \text{per cent CH}_4 \times 655) \div \text{ml. sample.}$$

$$\text{Cu. ft. CH}_4 \text{ per 1000 gal.} = 0.204 \times \text{ppm. CH}_4.$$

#### BIBLIOGRAPHY

- PARR, S. W. *The Analyses of Fuel, Gas, Water, and Lubricants*. McGraw Hill, New York (1932).  
 BUSWELL, A. M., AND LARSON, T. E. Methane in ground waters. *J. Amer. W. W. Assn.*, 29, 1978 (1937).  
 LARSON, T. E. Properties and determination of methane in ground waters. *J. Amer. W. W. Assn.*, 30, 1828 (1938).

## PART II

### SEWAGE, SEWAGE EFFLUENTS, INDUSTRIAL WASTES, POLLUTED WATERS, SLUDGES, AND MUDS \*

#### 1. Collection of Samples

##### A. REPRESENTATIVE SAMPLES

Only representative samples should be used for examination. The great variety of conditions under which collections must be made renders it impossible to prescribe a fixed procedure.

##### B. METHOD OF SAMPLING SEWAGE AND EFFLUENTS

The standard method comprises collection of a composite sample over a 24 hour period. In many cases it may be advisable to divide the sample to represent shift work or to extend it to cover the complete cycle of operation including all special, variable or periodic discharges at irregular intervals, including Saturday and Sunday clean-ups. This is particularly true with industrial wastes.

Individual portions should be taken in a wide-mouthed bottle having a diameter at the mouth of at least 35 mm. and a capacity of at least 120 ml. These should be collected each hour, in some cases each half hour, or even every five minutes, and

mixed at the end of the sampling period, or combined in a single bottle as collected (see preservation instructions below).

It is desirable, in many cases absolutely essential, to combine the individual samples in volumes proportionate to the volume of flow. A final volume of 2 to 3 liters is sufficient for sewage and effluents. The sample must be refrigerated so that it is chilled immediately to 3° to 4° C. and protected from light. Automatic sampling devices are available, but should not be used unless the sample is refrigerated to 3° to 4° C. Sampling devices, including bottles, should be cleaned daily of all growths of sewage organisms.

Under some conditions it may be necessary to collect "catch samples," in which case the results of analyses must be interpreted in the light of conditions prevailing at the time of collection.

##### C. PRESERVATION OF SAMPLES

Samples of sewage and effluents should be analyzed as soon as possible after collection. No single method of preservation is entirely satisfactory, and the preservative should be chosen with due regard to the determinations that are to be made.

\* A special committee of the Federation of Sewage Works Associations investigated methods for the chemical analysis of sewage and sewage sludge and as a result of a painstaking and thorough investigation, developed a manual for such examinations. This manual was approved by the Board of Control of the Federation of Sewage Works Associations which submitted it to the Joint Editorial Board of the 9th edition of Standard Methods for the Examination of Water and Sewage to be incorporated as Part II on standard methods for the chemical analysis of sewage, sewage effluents, industrial wastes, and polluted waters.

Formaldehyde affects so many of the determinations that its use is not recommended.

The preservation of samples for the determination of dissolved oxygen is discussed in Part II, Sec. 10, page 124.

When the sample cannot be analyzed immediately, it should be preserved as follows:

1. Samples for the determination of relative stability and biochemical oxygen demand *must be free from all preservatives*. When samples are composited, the samples or the composite sample should be kept in a cooler at 3° to 4° C. during the compositing period. In samples stored at room temperatures, the B.O.D. may drop 10 to 40 per cent in six hours. In some instances, the B.O.D. may rise.

2. Add 5 ml. of washed chloroform per liter and store on ice or at 4° C. This treatment preserves the oxygen consuming ( $\text{KMnO}_4$ ) value of the sewage. The chloroform should be washed in a separatory funnel three times with equal volumes of distilled water and then kept dry by storing it in dark bottles containing anhydrous calcium chloride. Dry chloroform, when exposed to light decomposes to form phosgene and hydrochloric acid.

3. Sufficient sulfuric acid (cp.) to produce 1500 parts per million acidity will preserve the nitrogen balance. This treatment will lower the suspended matter, settleable matter, and oxygen consumed values.

HATFIELD, W. D., AND PHILLIPS, GEO. E.  
Preservation of sewage samples. *W. Wks. and Sew.*, 88, 285 (1941).

## 2. Expression of Chemical Results

Analytical results shall be expressed in parts per million by weight, assuming that 1 liter of water, sewage or industrial waste weighs 1 kilogram. Industrial wastes of high specific gravity shall have the results expressed as milligrams per liter, with the specific gravity given. Only the significant figures should be recorded.

In reporting results on stream pollution or plant operation, and efficiencies, it is desirable to express the results on a weighted basis, thus including both the concentration and the volume of flow. These weighted results may be expressed, as quantity units according to the practice of the U. S. Public Health Service, as pounds per 24 hours, or as population equivalents (based on B.O.D.). Totals of the weighted units may be converted to the weighted average parts per million.

2.1. Calculation of quantity units.

Quantity units ( $\text{Q.U.}_1$ ) = ppm.  
 $\times 1000$  cu. ft. per sec.

Quantity units ( $\text{Q.U.}_2$ ) = ppm.  
 $\times \text{mgd.}$

Pounds per 24 hours = ppm.  
 $\times \text{mgd.} \times 8.34$ .

Pounds per 24 hours = ppm.  
 $\times \text{cfs.} \times 5.39$ .

Population equivalent = ppm. 5  
day B.O.D.  $\times \text{mgd.} \times (8.34 \div 0.17)$ .

## BIBLIOGRAPHY

STREETER, H. W., AND FROST, W. H. A study of the pollution and natural purification of the Ohio River, Sec. 5. Chemical Studies, U. S. Pub. Health Service, Bull 143, 128 (1924).

## BIBLIOGRAPHY

HOMEWOOD, R. T., AND RUFF, H. W. Ordinary chloroform as a sewage preservative increases oxygen consumed values. *Sew. Works J.*, 2, 251 (1930).



### 3. Physical Examination

#### A. TEMPERATURE

The temperature of sewages or effluents, at the time of sampling, shall be expressed in degrees centigrade to the nearest degree.

#### B. TURBIDITY

Turbidity is an expression of an optical approximation of the suspended matter, based on the similarity of interference to the passage of light rays through a sample when compared with standard samples of recorded turbidity. (See Part I, Sec. 6, page 10).

ml. of samples per 200 ml. dilution	0.91	0.67	0.5	0.33	0.22	0.13	0.1
Threshold number	220	300	400	600	900	1500	2000

#### 1. Apparatus

- 1.1. Jackson candle turbidimeter.
- 1.2. Photoelectric photometer.

#### 2. Procedure

2.1. Turbidity may be determined by using a Jackson candle turbidimeter, provided the suspended matter is finely divided. The procedure to be followed is given in Part I, Sec. 6, A, page 10.

2.2. Turbidity may also be measured in a photoelectric photometer that has been calibrated against prepared turbidity standards, which, in turn, have been calibrated against a Jackson candle turbidimeter.

#### C. COLOR

The color of sewage is largely dependent on industrial wastes and the age of the sewage. This determination is not, at present, on a quantitative basis; the determination, there-

fore, should be descriptive of the color and intensity.

#### D. ODOR

A considerable advance has been made in recent years in the quantitative determination of odors in water. This determination may be applied to sewages and industrial wastes.\*

#### 1. Procedure

The procedure for the odor determination is given in Part I, Sec. 8, page 16. For application to sewage and wastes, the dilution must generally be extended to higher ratios, as for example:

#### BIBLIOGRAPHY

- SPAULDING, C. H. The quantitative determination of odor in water. *Am. J. Pub. Health*, 21, 1038 (1931).
- FAIR, G. M., AND WELLS, W. F. The air dilution method of odor determination in water analysis. *J. Amer. W. W. Assn.*, 26, 1670 (1934).
- FAIR, G. M., AND MOORE, E. W. Measurement of intensity and concentration of odors associated with sewage treatment processes. *Sew. Works J.*, 7, 182 (1935). (See also Bibliography under Part I, Sec. 8, page 19.)

### 4. Ammonia Nitrogen

Because organic nitrogen in unsterilized sewage is progressively ammonified by saprophytic bacteria, the determination of ammonia or organic nitrogen must be made within 6 hours after collection of the sample, or the sample must be preserved with an excess of 1500 ppm.  $H_2SO_4$ .

Direct Nesslerization is preferable to distillation as it avoids errors from hydrolysis of urea and other organic compounds.

## A. DIRECT NESSLERIZATION

1. *Reagents*

1.1. Zinc sulfate. Dissolve 100 g. of  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  in ammonia-free distilled water and make up to 1 liter. Copper sulfate may be substituted for zinc sulfate if no hydrogen sulfide is present in the sample being examined.

1.2. Sodium hydroxide. Dissolve 250 g. of NaOH in ammonia-free distilled water and make up to 1 liter.

1.3. Ammonia-free water. (a) Ammonia-free water may be made by redistillation of distilled water that has been treated with bromine and allowed to stand overnight. (b) For most work the ammonia may be removed from ordinary distilled water by shaking with Folin's ammonia permittit. Traces of magnesium, however, in some distilled water causes cloudy Nessler tubes. Cloudy tubes may be prevented by the use of Rochelle salt solution (1.4).

1.4. Potassium and sodium tartrate (Rochelle salt) solution. Dissolve 500 g. of cp. Rochelle salt ( $\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$ ) in 1 liter of water. Boil off 200 ml. or until free from ammonia. Cool and restore volume with ammonia-free distilled water.

1.5. Standard ammonium chloride solution. For the stock solution dissolve 3.818 g. of  $\text{NH}_4\text{Cl}$  in ammonia-free distilled water and dilute to 1 liter. From this stock solution prepare the standard solution by diluting 10 ml. of this stock solution to 1 liter with ammonia-free distilled water: 1 ml. contains 0.01 mg. of nitrogen.

1.6. Nessler reagent. Dissolve 61.75 g. of potassium iodide (KI) in

250 ml. of ammonia-free water, and add a cold solution of mercuric chloride ( $\text{HgCl}_2$ ) which has been saturated by boiling with excess of the salt. Pour in the mercuric chloride solution cautiously, and add an amount just sufficient to make the color a permanent bright red. With a little practice the exact depth of color can be easily duplicated. It will take a little over 400 ml. of the mercuric chloride solution to reach this end point. Dissolve the red precipitate by adding exactly 0.75 g. of KI. Then add 150 g. of KOH dissolved in 250 ml. of water. Make up to 1 liter. Mix thoroughly and allow the precipitate formed to settle. Pour off the supernatant liquid. Mercuric chloride increases the sensitiveness of Nessler reagent and potassium iodide decreases it.

2. *Procedure*

Add 1 ml. of zinc sulfate solution (1.1) to 100 ml. of the sample; mix thoroughly, add 0.4 to 0.5 ml. of NaOH soln. (1.2) to obtain a pH of 10.5, again mix and clarify by centrifuging or filtering through filter paper, discarding the first 25 ml. of filtrate.

Dilute 5 ml. or less of the clarified liquor or filtrate to the mark in a 50 ml. Nessler tube with ammonia-free water. Add 1 or 2 drops of Rochelle salt solution (1.4) to prevent cloudy tubes. Nesslerize by adding 1 ml. of Nessler's reagent and after 10 minutes compare with standards.

Prepare a series of 16 Nessler tubes containing the following volumes of standard ammonium chloride solution, diluted to 50 ml. with ammonia-

free water: namely, 0.0, 0.2, 0.4, 0.7, 1.0, 1.4, 1.7, 2.0, 2.5. The standards will contain 0.01 mg. of nitrogen for each 1 ml. of the standard solution. Add 1 ml. of Nessler reagent (1.6) to each tube and allow ten minutes for color formation.

ppm. ammonia nitrogen =  $(\text{ml. NH}_4\text{Cl standard} \times 10) \div \text{ml. of Nesslerized sample.}$

ppm. ammonia nitrogen =  $(\text{mg. of N in standard} \times 1000) \div \text{ml. of Nesslerized sample.}$

(Note: A correction for the reagents added for clarification of the sample is not necessary unless amounts larger than directed are used.)

## B. PREPARATION OF PERMANENT STANDARDS

### 1. Reagents

1.1. Potassium chloroplatinate ( $\text{K}_2\text{PtCl}_6$ ). Dissolve 2 g. of the salt in 300 to 400 ml. of distilled water, add 100 ml. of concd. HCl and dilute to 1 liter with distilled water.

1.2. Cobaltous chloride,  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ . Dissolve 12 g. of dry crystals in 200 ml. of distilled water, add 100 ml. of concd. HCl and dilute to 1 liter.

### 2. Procedure

Permanent standards may be prepared with reagents 1.1 and 1.2 according to the directions given in Part I, Sec. 32, B, page 67, omitting such values as are not needed in sewage examination. These standards may be used for several months if protected from dust. Comparisons must be made at least 10 minutes after the sample has been Nesslerized. (Thirty minutes may be required for very faint colors to form.)

The values given in the table are approximate; actual equivalents of the standards thus prepared will differ with the quality of the Nessler reagent and the color sensitiveness of the analyst's eye. They should be compared with Nesslerized ammonia standards and the tints modified as necessary. Such comparison should be made for each newly prepared Nessler solution and checked by each analyst.

## BIBLIOGRAPHY

PHELPS, E. B. A critical study of the methods in current use for the determination of free and albuminoid ammonia. *Pub. Health Papers and Rpts. Am. Pub. Health Assn.*, 29, 354 (1903); *J. Inf. Dis.*, 1, 327 (1904).

## C. DISTILLATION METHOD

### 1. Apparatus

1.1. Distillation shall be carried on in a glass flask with a vertical condenser, so arranged that the distillate shall drop directly from the block tin or aluminum tube into the receiving vessels.

1.2. Nessler tubes. The Nessler tubes (50 ml. capacity) shall conform to the specification in Part I, Sec. 2, C, page 4.

### 2. Reagents

2.1. 0.5 M phosphate buffer solution. Dissolve 14.3 g. of monobasic potassium phosphate ( $\text{KH}_2\text{PO}_4$ ) and 90.15 g. of dibasic potassium phosphate ( $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$ ) in ammonia-free distilled water and make up to 1 liter.

### 3. Procedure

Steam out the still until free from ammonia. Place a portion of the sam-



ple (10 to 100 ml.) in an 800 ml. Kjeldahl flask. Add 25 ml. of the 0.5 M phosphate buffer solution, which keeps the pH of the distillation mixture at 7.4 during the distillation; dilute with 200 ml. of ammonia-free water and distill 200 ml.

Dilute an aliquot portion of the distillate with ammonia-free water to 50 ml. in a Nessler tube, Nesslerize and compare with standards as directed in Part II, 4, A, 2, page 116, or with permanent standards (Part II, 4, B, page 117).

To calculate the amount of ammonia nitrogen present, let  $R$  = the reading of ammonia standard expressed in ml. of  $\text{NH}_4\text{Cl}$  solution used, let  $S$  = ml. of sample taken for distillation and let  $d$  = ml. of distillate taken for Nesslerization.

$$\text{ppm. ammonia nitrogen} = [2000 \times R] \div [S \times d].$$

If permanent standards are used for comparison, reference must be made to the  $\text{NH}_4\text{Cl}$  equivalent of the standard matched, if the above equation is used.

## BIBLIOGRAPHY

JOHNSON, G. A. *Report on Sewage Purification at Columbus, Ohio, Made to the Chief Engineer of the Board of Public Service*, p. 47 (1905).

FOOTE, M. E., AND NICHOLS, M. S. The effect of hydrogen ion concentration on the distillation of free ammonia nitrogen from sewage and trade wastes. *Sew. Works J.*, 4, 37 (1932).

## 5. Organic Nitrogen

The Kjeldahl method, using copper sulfate as a catalyst and potassium or sodium sulfate to raise the boiling point of the sulfuric acid, is recommended as the standard procedure.

In most sewages, however, the addition of potassium or sodium sulfate may be eliminated from the digestion procedure. Direct Nesslerization of the digestate is satisfactory for plant control and field work, but distillation into standard acid or boric acid is necessary where accuracy is desired.

### 1. Reagents

1.1. Phosphate buffer solution. See Part II, Sec. 4, C, 2, page 117.

1.2. Concentrated sulfuric acid, low in nitrogen.

1.3. Copper sulfate. Dissolve 100 g.  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in ammonia-free water and dilute to 1 liter.

1.4. Sodium hydroxide. Dissolve 500 g. of  $\text{NaOH}$  in ammonia-free water and dilute to 1 liter.

1.5. Boric acid solution. Dissolve 40 g. of cp.  $\text{H}_3\text{BO}_3$  in 1 liter of ammonia-free distilled water.

1.6. Sulfuric acid 0.05 N solution. One ml. is equivalent to 0.7 mg. of nitrogen. Other strengths of standard acid may be used; for example 0.02 N gives greater accuracy and 0.0714 N reads directly with a factor of 10 when a 100 ml. sample is used.

1.7. Methyl red indicator.

### 2. Procedure

Measure a quantity, 100 ml. or more, of sample into an 800 ml. Kjeldahl flask, to which has been added 25 ml. of the phosphate buffer solution. Boil or distill off the free ammonia.

Add 10 ml. of concd.  $\text{H}_2\text{SO}_4$  and 1 ml. of  $\text{CuSO}_4$  soln. Digest by boiling in a hood for 20 to 30 minutes after the digestate has become clear. Cool

and add about 250 ml. of ammonia-free water.

Make alkaline with sodium hydroxide using the blue copper precipitate as an indicator, and distill into 50 ml. of  $\text{H}_3\text{BO}_3$  soln., until about 200 ml. of distillate has been obtained. (Bumping may be reduced with zinc or "boiling chips.")

Add 3 drops of methyl red indicator and titrate the ammonia with 0.05 N  $\text{H}_2\text{SO}_4$ , matching the end point with that of a blank containing the same amounts of boric acid and indicator diluted to the same volume with carbon dioxide-free distilled water.

A blank should be run on the reagents used and necessary corrections made. Distillation into standard acid (0.05 N) and back titration with standard base (0.05 N) may be used if preferred, but the boric acid procedure is simpler.

$\text{ppm. organic nitrogen} = \text{ml. of } 0.05 \text{ N acid} \times 0.7 \times (1000 \div \text{ml. of sample used}).$

#### BIBLIOGRAPHY

- KJELDAHL, J. A new method for the determination of nitrogen in organic matter. *Zeit. Anal. Chem.*, **22**, 366 (1883).
- PHELPS, E. B. The determination of organic nitrogen in sewage by the Kjeldahl process. *J. Infect. Dis. Suppl.*, **1**, 255 (1905).
- JERREL, T. D. Substitution of sodium sulfate for potassium sulfate in the Kjeldahl Gunning Arnold method for the determination of ammonia in fertilizers. *J. Assn. Off. Agr. Chem.*, **3**, 304 (1920).
- MEEKER, ED. W., AND WAGNER, E. C. Titration of ammonia in presence of boric acid. *Ind. Eng. Chem. Anal. Ed.*, **5**, 413 (1933).
- BRECHER, C. A new method for titrating ammonia in the micro-Kjeldahl determination. *Wien. Klin. Wchnschr.* **49**, 1228 (1936); *Chem. Abs.*, **31**, 3318 (1937).
- WAGNER, E. C. Titration of ammonia in the presence of boric acid. *Ind. Eng. Chem., Anal. Ed.*, **12**, 771-2 (1940).

## 6. Total Kjeldahl Nitrogen

This determination includes ammonia and organic nitrogen, but does not include nitrite and nitrate nitrogen.

### 1. Procedure

Measure 100 ml. or more of sample into an 800 ml. Kjeldahl flask and proceed directly as in organic nitrogen, Part II, Sec. 5, 2, page 118.

#### BIBLIOGRAPHY

(See Bibliography, Part II, Sec. 5.)

## 7. Nitrate Nitrogen

The following simplified reduction method with direct Nesslerization is recommended as the standard procedure for sewages and effluents. The phenoldisulfonic acid method is also included as a standard method. In strong sewages urea may hydrolyze to ammonia and indicate nitrates which are not present. In oxidized effluents, however, this error is remote.

### A. REDUCTION METHOD

#### 1. Reagents

1.1. Sodium or potassium hydroxide. Dissolve 250 g. of the hydroxide low in nitrogen in 1.25 liter of distilled water. Add several strips of aluminum foil and allow the evolution of hydrogen to continue overnight. Concentrate the solution to 1 liter by boiling.

1.2. Aluminum foil. Use strips of 30 gage pure aluminum sheet about 10 cm. long, 6 mm. wide, and 0.33 mm. thick, weighing about 0.5 g.

#### 2. Procedure

2.1. For normal sewages. Add 2 ml. of nitrate-free NaOH (1.1) to 100

ml. of sample and concentrate, by boiling in a casserole, to about 20 ml. to remove free ammonia. Rinse this concentrated sample into a 50 ml. Nessler tube (or graduated test tube), make up to the mark with ammonia-free water, add one strip of aluminum foil and cover the tube with a loosely fitting stopper (or Bunsen valve) to protect the solution from dust.

In laboratories where ammonia fumes are present in quantities, the ammonia trap described in Part I, Sec. 35, B, 2, page 71, should be used in place of the loosely-fitting stopper. Allow the reduction to take place at 20° C. or above for 6 hours, or overnight.

After reduction, Nesslerize 1 to 5 ml. of the clear reduced liquid (after dilution to 50 ml. with ammonia-free water) and compare with ammonia standards.

To prevent cloudy tubes, if such occur, pour the reduced liquid into a large test tube which contains 0.5 ml. of 10 per cent  $\text{CuSO}_4$  or  $\text{ZnSO}_4$  soln. and centrifuge or filter, discarding the first 25 ml. of filtrate. Nesslerize 1 to 5 ml. of the filtrate.

(1) ppm.  $\text{NO}_3^-$  nitrogen +  $\text{NO}_2^-$  nitrogen  
 = (ml. of  $\text{NH}_4\text{Cl}$  standard  $\times$  5)  $\div$  ml. soln.  
 Nesslerized.

or

(2) ppm.  $\text{NO}_3^-$  nitrogen +  $\text{NO}_2^-$  nitrogen  
 = (mg. N in standard  $\times$  500)  $\div$  ml. soln.  
 Nesslerized.

(3) By subtracting the nitrite nitrogen from (1) or (2) the nitrate nitrogen is obtained.

**2.2. For industrial wastes.** In some industrial wastes and oxidized effluents from trade waste treatment, very high nitrite concentrations may

be encountered. The nitrite correction may be eliminated by adjusting the reaction of the sample to pH 7.5, adding  $\text{NH}_4\text{Cl}$  to make a concentration of 2000 ppm. and evaporating to dryness.

Take up the residue in 100 ml. of distilled water, add 5 ml. of 40 per cent nitrate-free  $\text{NaOH}$  and boil down to about 25 ml. to expel ammonia. Proceed with the reduction and Nesslerization as described above and report results as nitrate nitrogen, the nitrites having been volatilized on evaporation with the ammonium chloride.

## B. PHENOLDISULFONIC ACID METHOD

Chlorides affect this determination by reducing the nitrate nitrogen. This reduction is proportional to both the chloride and nitrate concentrations. The chlorides should be removed by treatment with silver sulfate. (See Part I, Sec. 35, A, 2, page 70.)

### 1. Reagents

**1.1. Aluminum hydroxide.** Dissolve 125 g. ammonium or potassium alum in 1 liter of water. Heat to 60° C. and add slowly, while stirring, 55 ml. of concd.  $\text{NH}_4\text{OH}$ . Allow this mixture to stand approximately 1 hour, and then transfer to an 8 liter bottle and wash by successive decantations with distilled water until free from chloride, ammonia, nitrite, and nitrate.

**1.2. Activated carbon.** Prepare a nitrate standard containing 0.01 mg. of nitrate nitrogen per 100 ml. of solution. To 50 ml. of this alkaline standard add 1 g. of activated carbon



and stir. Filter and compare with the untreated portion. If there is no reduction of color the activated carbon is satisfactory for use.

1.3. Phenoldisulfonic acid. (Part I, Sec. 35, A, 1, page 69.)

1.4. Potassium hydroxide, 12 N. (Part I, Sec. 35, A, 1, page 69.)

1.5. Standard nitrate solution. (Part I, Sec. 35, A, 1, page 69.)

1.6. Zinc sulfate. 100 g.  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  in 1 liter. (Part II, Sec. 4, A, 1, page 116.)

1.7. Sodium hydroxide. 500 g. NaOH in 1 liter. (Part II, Sec. 4, A, 1, page 116.)

## 2. Procedure

2.1. For sewages containing little color. Clarify sewage with  $\text{ZnSO}_4$  (1.6) and NaOH (1.7) as described for the determination of ammonia nitrogen, Part II, Sec. 4, page 115.

Evaporate 1 to 25 ml. of the clarified filtrate to dryness without spattering and thoroughly moisten with 1 ml. of phenoldisulfonic acid (1.3).

Dilute to 20 ml. with distilled water and make alkaline with KOH soln. (1.4) (5 or 6 ml.) until a maximum yellow color is obtained. Dilute and compare with color standards prepared as described in Part I, Sec. 35, A, 2, page 70.

2.2. For highly colored sewages. Highly colored sewages require more complete methods of clarification and decolorization.

To 50 ml. of sewage or effluent add about 0.5 g. of activated carbon (1.2) and 1 ml. of  $\text{Al}(\text{OH})_3$  cream (1.1) mix and filter. Discard the first portion of the filtrate.

Evaporate, 1 to 25 ml. of the fil-

trate, to dryness on a water bath. Thoroughly moisten the residue with 1 ml. of phenoldisulfonic acid and dilute to 20 ml. with distilled water.

Make the solution alkaline with 5 to 6 ml. of KOH (1.4) until a maximum yellow color is obtained.

Treat with 0.5 g. activated carbon and filter into a Nessler tube. Make the volume up to the mark with distilled water and compare with standards as prepared in Part I, Sec. 35, A, 2, page 70.

$\text{ppm. NO}_2^- \text{ nitrogen} = (\text{ml. of standard} \div \text{ml. of sample}) \times 10.$

## BIBLIOGRAPHY

NELSON, G. H., LEVINE, MAX, AND BUCHANAN, J. H. Elimination of corrections for nitrites in nitrate determinations. *Ind. Eng. Chem., Anal. Ed.*, 4, 56 (1932).

## 8. Nitrite Nitrogen

Determination of the nitrite content shall be made on fresh samples because conversion of nitrite into nitrate or into ammonia by bacterial action proceeds uninterruptedly. (See Part I, Sec. 1, B, page 1.) Results are to be reported in terms of the nitrogen equivalent.

### 1. Reagents

1.1. Sulfanilic acid solution. Dissolve 8 g. of cp. sulfanilic acid in 1 liter of 5 N acetic acid (sp. gr. 1.041, or 285 ml. glacial acetic acid diluted to 1 liter). This is practically a saturated solution.

1.2.  $\alpha$ -Naphthylamine-acetate solution. Dissolve 5 g. of solid  $\alpha$ -naphthylamine in 1 liter of 5 N acetic acid (285 ml. of glacial acetic acid diluted to 1 liter). Filter the solution periodically through washed absorbent

cotton to remove decomposition products and store in dark bottle. The reagent is stable only for 1 or 2 months.

1.3. Sodium nitrite stock solution. Dissolve 0.497 g. of sodium nitrite in 1 liter of nitrite-free distilled water.

1.4. Sodium nitrite standard solution. Dilute 100 ml. of solution (1.3) to 1 liter, then dilute 50 ml. of this solution to 1 liter with sterilized nitrite-free distilled water, add 1 ml. of chloroform, and preserve in a sterilized bottle. One ml. is equivalent to 0.0005 mg. N or 0.001642 mg.  $\text{NO}_2$ .

1.5. Aluminum hydroxide (Part II, Sec. 7, B, 1.1, page 120).

1.6. Nitrite free water. Add 1 ml. concd.  $\text{H}_2\text{SO}_4$  and 0.2 ml. of  $\text{MnSO}_4$  soln. (Part II, Sec. 10, D, 1.1, page 127) to 1 liter of distilled water and make pink with 1 to 3 ml. of 0.0125 N  $\text{KMnO}_4$  (Part II, Sec. 9, 1.3, page 123). After 15 minutes decolorize with 0.0125 N  $(\text{NH}_4)_2\text{C}_2\text{O}_4$  (Part II, Sec. 9, 1.2, page 123).

## 2. Procedure

Place in a standard Nessler tube 0.1 to 10 ml. of the sample, clarified if necessary with  $\text{ZnSO}_4$  and  $\text{NaOH}$  as described for ammonia nitrogen (Part II, Sec. 4, A, 2, page 116) and dilute to 50 ml. At the same time prepare in Nessler tubes a set of standards by diluting to 50 ml. with nitrite-free water (1.6) various amounts of standard nitrite.

The following volumes of standard solution (1.4) are suggested: 0.0, 0.1, 0.2, 0.4, 0.7, 1.0, 1.4, 1.7, 2.0 and 2.5 ml. Permanent standards may be

prepared from fuchsin—according to Part I, Sec. 36, 1.6 and 2, page 72.

Add 1 ml. of sulfanilic acid solution and 1 ml. of the  $\alpha$ -naphthylamine acetate solution to the sample and to each standard. Mix thoroughly and allow to stand 10 minutes; then compare the sample with the standards. Do not allow the sample to stand more than 30 minutes before making the comparison.

ppm. nitrite nitrogen = ml. standard  $\times 0.5 \div$  ml. of sample.

## BIBLIOGRAPHY

- LOS VAY, M. L. Nitrous acid in the saliva and in exhaled air. *Bull. de la Soc. Chem., Ser. 3*, 2, 388 (1889).  
 WESTON, R. S. Notes on the determination of nitrogen as nitrites in waters. *J. Am. Chem. Soc.*, 27, 281 (1905).  
 REINDOLLAR, W. F. Nitrite nitrogen standards. *Ind. Eng. Chem., Anal. Ed.*, 12, 325 (1940).

## 9. Oxygen Consumed from Permanganate

The oxygen consumed from permanganate is that amount used by the sample when digested for 30 minutes in a boiling water bath with a definite strength of acid or alkaline permanganate.

Since the carbon and not the nitrogen, in organic matter is oxidized by potassium permanganate, oxygen consumed is sometimes erroneously considered as indicating the amount of carbonaceous organic matter present. The determination, however, indicates only a part of the carbon, the proportion varying in different samples because the carbon in nitrogenous organic matter is not so readily oxidized as that in carbonaceous organic matter. The test does not directly

differentiate the carbon present in unstable organic matter from that in fairly stable organic matter. It is of value in estimating the strength of certain trade wastes and sewages when the biochemical oxygen demand (B.O.D.) cannot be determined, or in conjunction with the determination of the B.O.D.

The concentrations of potassium permanganate and sulfuric acid, and the temperature of digestion used in the procedure, control the final oxygen consumed results and, therefore, the technic must be identical with the standard procedure if the results are to be comparable.

Nitrites, ferrous iron, sulfide and other oxidizable substances reduce permanganate; therefore, corrections for them should be made. Direct titration of the acidified sample with the permanganate solution in the cold, to an end point permanent for 3 minutes, serves this purpose.

### 1. Reagents

1.1. Dilute sulfuric acid. Add 1 volume of cp.  $\text{H}_2\text{SO}_4$  to 3 volumes of distilled water. This solution may be oxidized with permanganate but this is not necessary, because a blank determination on all reagents must be run.

1.2. Ammonium oxalate solution. Dissolve 0.8880 g. of cp.  $(\text{NH}_4)_2\text{C}_2\text{O}_4 \cdot \text{H}_2\text{O}$  in 1 liter of distilled water. One ml. is equivalent to 0.1 mg. of oxygen. This solution is *not stable* and should be prepared each month. It may be stabilized by making the solution 0.1 N with ammonium hydroxide.

1.3. Standard potassium permanganate. Dissolve 0.4 g. of cp.  $\text{KMnO}_4$

in 1 liter of distilled water. Add 10 ml. of this solution and 10 ml. of the dil.  $\text{H}_2\text{SO}_4$  to 100 ml. of distilled water in an Erlenmeyer flask, and digest 30 minutes in boiling water. Add 10 ml. of the standard  $(\text{NH}_4)_2\text{C}_2\text{O}_4$  soln., and then titrate to a pink coloration with the standard  $\text{KMnO}_4$  soln. This destroys the oxygen consuming capacity of the distilled water. Now add 10 ml. of the  $(\text{NH}_4)_2\text{C}_2\text{O}_4$  soln. and titrate with the  $\text{KMnO}_4$  soln. Adjust the permanganate solution so that 1 ml. is equivalent to 1 ml. of the oxalate solution or to 0.1 mg. of available oxygen. Preserve this solution in a dark glass bottle.

1.4. Thirty-three per cent sodium hydroxide solution. Dissolve 500 g. of  $\text{NaOH}$  in 1 liter of distilled water. Settle and decant the clear supernatant liquid.

1.5. Manganous sulfate solution. Dissolve 67 g. of  $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$  in 500 ml. of distilled water. Add 138 ml. of 85 per cent  $\text{H}_3\text{PO}_4$  and 130 ml. of concd.  $\text{H}_2\text{SO}_4$  and dilute to 1 liter.

1.6. Ferrous ammonium sulfate solution. Dissolve 4.9 g. of cp. ferrous ammonium sulfate in 500 ml. of recently boiled and cooled distilled water. Add 8 ml. of concd.  $\text{H}_2\text{SO}_4$  and dilute to 990 ml. with recently boiled and cooled distilled water.

Add 10 ml. of this solution to 100 ml. of distilled water containing 25 ml. of manganous sulfate solution (1.5) and titrate with the standard potassium permanganate. Adjust the ferrous ammonium sulfate solution so that 1 ml. is equivalent to 1 ml. of standard permanganate. The solution must be checked each time it is used.



## 2. Procedure

2.1. Size of sample. If water low in pollution is being examined, use a 100 ml. portion. With sewage, dilute from 1 to 10 ml. of the sample to 100 ml. with distilled water. With sewage effluents or polluted waters, dilute 25 to 50 ml. to 100 ml. with distilled water. In all cases, the volume of sample plus diluent must be 100 ml., and furthermore, the quantity of sample must be such that an excess of not less than 5 ml. of the permanganate remains after digestion.

2.2. For normal sewage effluents and polluted waters. To the sample in an Erlenmeyer flask, diluted to 100 ml. with distilled water if required, add 10 ml. of the  $\text{KMnO}_4$  soln. and 10 ml. of the dil.  $\text{H}_2\text{SO}_4$  soln. Digest for 30 minutes in a boiling water bath, with the flask set so that the entire amount of sample plus reagent is submerged. Add 10 ml. of the  $(\text{NH}_4)_2\text{C}_2\text{O}_4$  soln. and titrate back with standard  $\text{KMnO}_4$  soln. to a faint pink color. Digest a blank made up of 100 ml. of distilled water and the reagents used, discharge the color with oxalate and titrate with the permanganate. Subtract the proportionate number of ml. of permanganate solution used by the distilled water from that used by the sample.

ppm. oxygen consumed = net ml. of  $\text{KMnO}_4 \times 100 \div \text{ml. of sample.}$

2.3. For brines or water high in chloride. Digestion in alkaline solution is preferable for brines or water high in chloride. Place 100 ml. of the sample, or a smaller quantity diluted to 100 ml. in a flask. Add 0.5 ml. of 33 per cent  $\text{NaOH}$  soln. (1.4)

and 10 ml. of standard permanganate (1.3), and digest 30 minutes in boiling water bath.

Remove flask, add 25 ml. of  $\text{MnSO}_4$  soln. (1.5) and 10 ml. of  $\text{FeSO}_4 \cdot (\text{NH}_4)_2\text{SO}_4$  soln. (1.6). Cool to room temperature and titrate with the standard  $\text{KMnO}_4$  soln.

2.4. For strong sewage. A standard permanganate solution of 10 times the above strength may be used with strong sewages. This usually gives higher results because of the stronger oxidizing capacity of the concentrated permanganate solution. On oxidized effluents the stronger permanganate oxidizes more of the stable organic matter and gives results many times greater than the B.O.D. On some wastes and digested liquors the results will be lower, rather than higher.

## BIBLIOGRAPHY

- TROMMSDORFF, H. Determination of organic substances. *Z. Anal. Chem.*, 8, 344 (1869).  
 TIDY, C. M. Processes for determining the organic purity of potable waters. *J. Chem. Soc.*, 35, 46 (1879).  
 BENSON, H. K., AND HICKS, J. F. G., JR. Proposed modification of oxygen consumed method for determination of sea water pollution. *Ind. Eng. Chem., Anal. Ed.*, 3, 30 (1931).  
 TREADWELL, F. P., AND HALL, W. T. *Analytical Chemistry*, Ed. 8, Vol. 2, 543, J. Wiley & Sons, New York (1935).

## 10. Dissolved Oxygen

### A. COLLECTION OF SAMPLES

Sample bottles of the type commonly used in oxygen demand determinations (240 to 380 ml.) are very satisfactory. At the start of an investigation it may nevertheless be desirable, for convenience and uni-

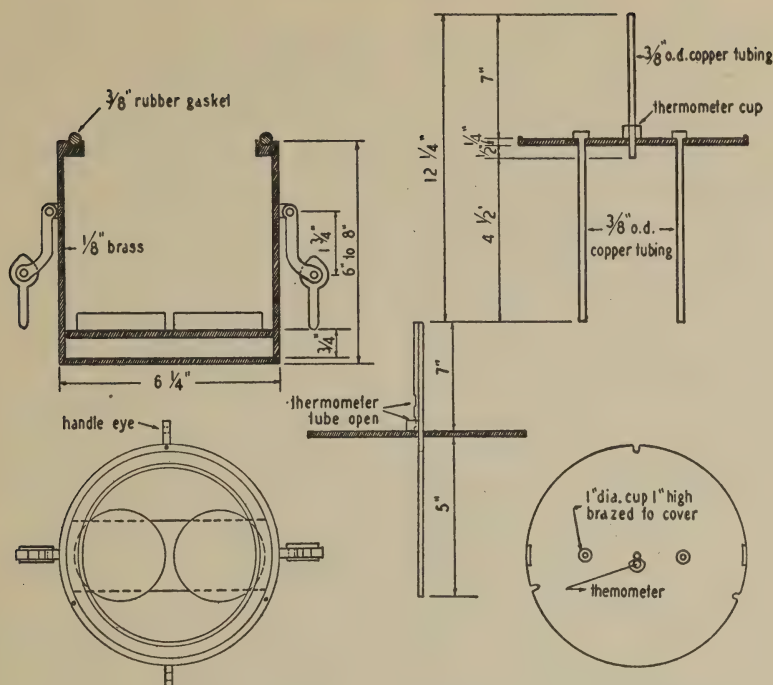


FIG. 11. DISSOLVED OXYGEN AND BIOCHEMICAL OXYGEN DEMAND SAMPLER ASSEMBLY.

formity, to select a 250 ml. glass stoppered bottle (8 ounce tincture).

In general, the sampling arrangements should be such as to insure at least a three-fold displacement of the liquid in the sampling bottle without entrainment of air bubbles. In sampling from streams, ponds, or tanks of moderate depth, this may be accomplished by the use of apparatus of the type shown in Fig. 11. The cover must be made air tight with gaskets to prevent the entrance of water around the cover.

It will be noted that the sample bottles begin to fill as soon as the above mentioned sampling devices are submerged. If a sample is required from a considerable depth, it may be desirable to use a sampler provided

with a valve release. The temperature of the sampled water should be recorded to the nearest degree centigrade, or more precisely as desired.

When special dissolved oxygen samples cannot be analyzed immediately the samples may be preserved for a 24 hour period, as follows:

To the 250–300 ml. D.O. bottle containing the sample, add 0.7 ml. of concd.  $\text{H}_2\text{SO}_4$  and .1 ml. of a 2 per cent sodium azide solution. Stopper and store at the temperature of collection, or water-seal in the  $20^\circ \text{C}$ . incubator, until the analysis can be made. Then continue with the Winkler procedure using 1 ml. of  $\text{MnSO}_4$  soln., 3 ml. of alkaline-iodide solution and 2 ml. of concd.  $\text{H}_2\text{SO}_4$  for the final acidification.

## B. SELECTION OF A METHOD OF PROCEDURE

Huge errors may be introduced in dissolved oxygen work (and also in oxygen demand work) by the neglect of proper precautions in the presence of interfering substances such as nitrites, ferrous salts, organic matter, sulfites, etc., or by an improper application of the various modifications of the Winkler method which are designed to overcome this type of interference.

In addition to the Winkler method, which should be used in determining the dissolved oxygen of relatively pure waters, the four modifications are:

The Alsterberg or sodium azide procedure for use in the presence of appreciable amounts of nitrites.

The Rideal-Stewart or permanganate modification for use in the presence of ferrous salts and nitrites.

The alkaline-hypochlorite modification for use in the presence of sulfites, thiosulfates, polythionates, free chlorine, or hypochlorites.

The alum flocculation modification for use in the presence of suspended solids which interfere with the determination of D.O.

The detailed application of these modifications of the Winkler method is described in the following pages.

## C. STANDARDIZATION OF THIOSULFATE SOLUTIONS

### 1. Reagents

1.1. Starch solution. Approx. 0.5 per cent. Grind 5 or 6 g. of potato starch in a mortar with a small quantity of water. Pour into 1 liter of

boiling water, stir and allow to settle overnight. Use the clear supernatant. This solution may be preserved with 1.25 g. salicylic acid per liter.

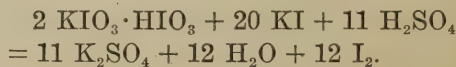
1.2. Dilute acid for standardization. 3.6 N  $\text{H}_2\text{SO}_4$  approximately. (One volume of concentrated sulfuric acid poured into 9 volumes of water).

1.3. Potassium or sodium iodide crystals. Free from iodates.

1.4. Stock sodium thiosulfate solution. Dissolve 24.82 g. of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  in 1 liter of distilled water; this corresponds to a 0.1 N solution. The solution may be preserved: (1) by chloroform (5 ml. per liter after making up to the mark), or (2) by the addition of 0.4 g. of NaOH per liter.

1.5. Standard sodium thiosulfate solution. 0.025 N. Prepared by diluting 250 ml. of stock thiosulfate solution (0.1 N) to 1 liter or by weighing out 6.205 g. of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  and making up to 1 liter with *freshly boiled distilled water*. Standard sodium thiosulfate solutions may be preserved over long periods with 0.4 g. of NaOH per liter.

1.6. Potassium bi-iodate solution. A stock solution of strength equivalent to that of the 0.1 N thiosulfate contains 3.250 g. of  $\text{KIO}_3 \cdot \text{HIO}_3$  per liter in accordance with the following reaction:



A bi-iodate solution equivalent to the 0.025 N thiosulfate solution contains 0.8124 g. of  $\text{KIO}_3 \cdot \text{HIO}_3$  per liter and may be prepared by diluting 250 ml. of the stock bi-iodate solution to 1 liter.



1.7. Potassium dichromate may be substituted for potassium bi-iodate if difficulty is experienced in securing the latter reagent. A solution of potassium dichromate equivalent to the 0.025 N sodium thiosulfate should contain 1.225 g. of  $K_2Cr_2O_7$  per liter.

## 2. Procedure

For the standardization dissolve approximately 5 g. of potassium iodide (free from iodate) in an Erlenmeyer flask with 100 to 150 ml. of distilled water and add 10 ml. of dil.  $H_2SO_4$  (1:10), followed by 40 ml. of a standard 0.025 N bi-iodate (or dichromate) solution. Set the mixture in the dark for 5 minutes, dilute to 200 ml. for the bi-iodate and 400 ml. for the dichromate and titrate the liberated iodine with the thiosulfate solution, adding starch toward the end of the titration when a pale straw color is reached. Exactly 40 ml. of thiosulfate should be required when the solutions under comparison are of equal strength. One ml. is equivalent to 0.2 mg. of oxygen.

## D. THE WINKLER METHOD

The Winkler method should be used in determining the dissolved oxygen content of relatively pure waters. This method for the determination of D.O. depends on the formation of a precipitate of manganous hydroxide in a glass-stoppered bottle completely filled with the water under examination. The oxygen dissolved in the water is rapidly combined with the manganous hydroxide, forming a mixture of higher oxides, which, on acidification in the presence of an iodide, releases iodine in a quantity chemi-

cally equivalent to the oxygen content of the sample. The liberated iodine is then titrated with a standard solution of sodium thiosulfate. Modifications of this general procedure for counteracting the effect of interfering substances are presented in this Sec., E, F, G, and H.

## 1. Reagent

1.1. Manganous sulfate solution. Dissolve 480 g. of  $MnSO_4 \cdot 4H_2O$  or 400 g. of  $MnSO_4 \cdot 2H_2O$  in distilled water, filter and make up to 1 liter. When uncertainty exists regarding the water of crystallization, a solution of equivalent strength may be obtained by adjusting the specific gravity of the solution to a value of 1.270 at 20° C. The manganous sulfate solution should liberate not more than traces of iodine when added to an acidified solution of potassium iodide. (This indicates the absence of manganic and ferric salts.)

1.2. Alkaline-potassium iodide reagent. 500 g. of NaOH (or 700 g. of KOH) and 135 g. of NaI (or 150 g. of KI) per liter. Potassium and sodium salts may be used interchangeably. The reagent should be practically free from carbonates. It should not give any color with starch when diluted and acidified.

1.3. Concentrated sulfuric acid. Specific gravity 1.83 to 1.84. The strength of this acid is about 36 N. Hence 1 ml. is equivalent to about 3 ml. of the alkaline iodide solution.

## 2. Procedure

The following procedure is applicable to the determination of dissolved oxygen in the absence of un-

stable forms of organic matter and of such readily oxidizable mineral substances as nitrites, ferrous salts, polythionates, etc.

The volume of Winkler reagents to be added is based on the assumption that bottles of the size recommended herein (250 ml.) are being used. With larger bottles (300 to 380 ml.), 2 ml. each of the Winkler reagents should be used. With smaller bottles (125 ml. or less), it will be sufficient to add 0.5 ml. each of the three Winkler reagents (manganous sulfate solution; alkaline-iodide solution; concentrated sulfuric acid).

2.1. Addition of reagents. Add 1 ml. of the  $\text{MnSO}_4$  soln. followed by 1 ml. of alkaline-iodide solutions well below the surface of the liquid.

Shake well, by inverting the bottle several times, so as to distribute the precipitate uniformly throughout the bottle.

As the precipitate settles readily, the dissolved oxygen in the upper part of the bottle will not be completely absorbed unless the period of shaking is suitably prolonged. The shaking should be repeated a second time. With sea water a 10 minute period of contact with the manganous hydroxide may be required.

Numerous forms of organic matter are capable of direct oxidation by dissolved oxygen at pH values of 12 or thereabouts (corresponding to the degree of alkalinity obtained when alkaline-iodide is added to a sample). For this reason the test should be carried through on individual samples when interference by organic matter is suspected and the period of alkalization should be reduced to the mini-

mum time consistent with the complete absorption of dissolved oxygen.

When 1 ml. of  $\text{MnSO}_4$  soln. is used with 250 ml. of sample it may safely be assumed that the absorption of dissolved oxygen will be practically complete if the precipitated manganous hydroxide is agitated continuously for 40 to 50 seconds. With 2 ml. of  $\text{MnSO}_4$  soln., a shorter period of shaking (20 to 25 seconds) is sufficient. If the sample is then acidified as soon as the precipitate has settled, accurate results will be obtained in the presence of organic matter in amounts corresponding to 1000 ppm. of either dextrose or peptone.

Larger amounts of organic materials may be present (say 5000 ppm. or 0.5 per cent of dextrose) without introducing any appreciable error, if the sample is acidified before the precipitate has settled (that is, immediately after shaking), suitable allowance being made for the slight loss of precipitate by displacement on adding the acid. This procedure should be followed with sulfite wastes.

2.2. Acidification. Acidify with 1 ml. of concd.  $\text{H}_2\text{SO}_4$ , by allowing the acid to run down the neck of the bottle.

To avoid the reduction of manganic salts by organic materials, it is desirable that the sample be shaken as soon as possible after the addition of acid when organic substances are present.

The liberated iodine diffuses slowly. It should be uniformly distributed throughout the bottle before withdrawing a part of the sample for titration.

2.3. Titration. In the absence of

ferric salts and certain forms of organic matter (acetic acid, for example), it may be possible to delay the titration for several days without introducing an appreciable error.

The titration may be delayed for one hour, even in the presence of 100 ppm. of ferric iron, if potassium fluoride has been added in the preliminary treatment. In the absence of fluorides, the titration should be performed immediately after the final acidification when the ferric iron content exceeds 10 ppm.

The titration may be delayed for several days, even in the presence of 100 ppm. of iron if phosphoric acid (4 ml. of 85 per cent  $\text{H}_3\text{PO}_4$ ) is substituted for sulfuric acid in the final acidification.

A correction for the loss of dissolved oxygen by displacement with the reagents should be applied to the amount measured out for titration. Thus, when 1 ml. each of the manganous sulfate and alkaline-iodide reagents are added to a 250 ml. bottle, the amount measured out for titration should be:  $(200 \times 250) \div (250 - 2) = 201.6$  ml. if it is desired to titrate 200 ml. of the original sample.

Titrate with 0.025 N thiosulfate until the amount of iodine remaining is equivalent to about 0.5 ml. of thiosulfate solution, corresponding to a pale straw color in the sample.

Add 1 or 2 ml. of freshly prepared starch solution and titrate rapidly to the first disappearance of the blue color. Subsequent recolorations owing to the presence of traces of iron salts or to the catalytic effect of nitrites, etc., should be disregarded.

When several samples are being

run at the same time, four or five aliquot portions may be poured out at one time if the titrations are not delayed provided that iron salts and nitrites are not present in more than traces.

The loss of iodine by diffusion into the air is appreciable if the oxygen content exceeds 7 parts per million, and the titration is unduly delayed.

2.4. Calculation. When the volume titrated is equivalent to 200 ml. of the original sample, the dissolved oxygen content in parts per million is numerically the same as the required volume of 0.025 N thiosulfate expressed in milliliters. In general,

$$\text{ppm. dissolved oxygen} = [\text{ml. of } 0.025 \text{ N } \text{Na}_2\text{S}_2\text{O}_3 \times 200] \div \text{ml. of sample titrated.}$$

#### E. THE ALSTERBERG OR SODIUM AZIDE MODIFICATION

The sodium azide modification of the Winkler process should be used when appreciable amounts of nitrites are present in the sample. As a rule nitrites will occasion erroneously high results when present in amounts exceeding 0.1 ppm. of nitrogen as nitrite. Nitrites, although absent at the start, may appear during incubation in oxygen demand tests and interference due to them may also be expected in dealing with the effluent from biological oxidation devices.

This method may be used by adding the sodium azide solution in a preliminary step following acid, or it may be added in combination with the alkaline-iodide Winkler reagent. The first method affords greater flexibility, while the latter has manipulative advantages for routine work.



The azide method is not recommended for samples containing more than 1 ppm. of ferrous iron, or for sulfite wastes, or any industrial wastes containing reducing or oxidizing materials.

With the addition of potassium fluoride preliminary treatment with azide is applicable in the presence of several hundred parts per million of ferric iron if the sample is titrated immediately following final acidification.

### 1. Reagents

1.1. Alkaline-iodide-sodium azide. 500 g. NaOH (or 700 g. KOH), 135 g. NaI (or 150 g. KI) and 10.0 g. NaN<sub>3</sub> per liter. Dissolve these compounds separately in small portions of distilled water. Mix the solutions of sodium hydroxide and iodide; dilute to 950 ml., cool to room temperature and then add slowly the sodium azide (10 g. in 40 ml. of water) with constant stirring to avoid local heating. Make up to one liter. (When the preliminary azide procedure is used a 2 per cent aqueous solution of sodium azide is required.)

### 2. Procedure

The procedure is identical with that of the Winkler method, except that under 2.1 the alkaline-iodide-sodium azide solution is used in place of the Winkler alkaline-iodide solution, and 1.5 ml. of concentrated sulfuric acid are recommended instead of 1 ml. for acidification.

## F. THE RIDEAL-STEWART OR PERMANGANATE MODIFICATION

The Rideal-Stewart modification of the Winkler method should be used

when appreciable amounts of *ferrous salts* are present in the sample. The method will also eliminate interference due to the presence of nitrites. One ppm. of ferrous iron will occasion an apparent loss of about 0.14 ppm. of dissolved oxygen. On the other hand, high results may be obtained in the presence of ferric salts owing to the liberation of iodine from iodides in the final step of the Winkler process, especially if the titration is delayed.

This method with the addition of potassium fluoride should be used on coal mine drainage and acid streams which may contain considerable quantities of ferrous and ferric salts.

The permanganate modification is not recommended as a corrective for interference due to relatively stable forms of organic matter, such as sugars, starches, etc., which are not readily acted upon by permanganate in the cold. A preliminary treatment with permanganate of samples containing such substances is not beneficial and may actually be detrimental. The permanganate modification fails in the presence of sulfite wastes, in suspensions of river mud, and in undiluted sewage.

### 1. Reagents

The following solutions should be prepared when the permanganate modification of the Winkler method is to be used as a preliminary treatment for the destruction of ferrous iron:

1.1. Potassium permanganate solution. Dissolve 6.32 g. of KMnO<sub>4</sub> in distilled water and make up to 1 liter.

1.2. Oxalate solution. Dissolve 2

g. of potassium oxalate  $(\text{COOK})_2 \cdot \text{H}_2\text{O}$  in 100 ml. of distilled water. One ml. of this oxalate solution will suffice for the neutralization of about 1.1 ml. of the permanganate solution. This solution may be stabilized by adding 0.4 g. NaOH per liter. An oxalic acid solution of equivalent strength (1.4 g. of  $(\text{COOH})_2 \cdot 2\text{H}_2\text{O}$  in 100 ml. of distilled water) may be used.

In the presence of appreciable amounts of iron salts, the following solutions will also be required:

1.3. Fluoride solution. Dissolve 40 g. of  $\text{KF} \cdot 2\text{H}_2\text{O}$  in distilled water and make up to 100 ml.

1.4. Phosphoric acid. 85 per cent  $\text{H}_3\text{PO}_4$ .

## 2. Procedure

All reagents, excepting the sulfuric acid should be added well below the surface of the liquid.

### 2.1. Addition of reagents.

2.1.1. Add 0.7 ml. of concd.  $\text{H}_2\text{SO}_4$ . It is important that the amount called for should not be exceeded. The 0.7 portion, for that reason, is best added with a graduated 1 ml. pipette.

2.1.2. Add enough permanganate solution (usually 1 ml.) to obtain a violet tinge which persists after shaking.

With samples whose behavior is fairly well known, the proper amount of permanganate may be added all at once.

With samples of unknown or variable composition, trial amounts (1 ml. or less) should be added with shaking and a suitable delay allowed between additions. When an additional

amount of permanganate appears necessary, it should be added while a faint violet tinge still persists (that is, before the appearance of brownish colorations due to intermediate oxides).

To avoid too great a dilution of the sample, a stronger solution of permanganate should be used when a large amount (5 ml. or more) of this reagent is required.

2.1.3. In practice, standing five minutes at ordinary temperatures after obtaining a permanent violet coloration is sufficient to reduce the concentrations of ferrous iron and nitrite to values which will not cause undue interference.

2.1.4. Potassium oxalate solution is added to destroy the excess of permanganate whenever the oxidation of the interfering substances (readily oxidizable materials) is judged complete.

As a trial amount which should not be greatly exceeded, it is recommended that 0.5 ml. of the 2 per cent oxalate solution be first added. If after standing for five minutes the color of permanganate still persists, an additional 0.5 ml. portion of oxalate soln. should be added. It is important that perfect decolorization be secured, as even a trace of brownish coloration will react with iodides on acidification, giving results which may be entirely too high.

In the presence of iron salts, decolorization is delayed to a surprising extent (24 hours under certain conditions) even when the correct amount of oxalate has been added. In order to hasten the decolorization when iron salts are known to be present, potas-

sium fluoride (2 ml. of 40 per cent solution) should be added along with the permanganate or at any other stage of the preliminary treatment.

For the reason that ferric salts in the presence of oxalates are readily reduced to the ferrous condition by light (very rapidly so in direct sunlight), the decolorization must proceed in the dark when iron salts are present in amounts greater than 1 ppm.

2.1.5. When the permanganate is fully decolorized, add 1 ml. of  $\text{MnSO}_4$  soln. followed by 3 ml. of alkaline-iodide reagent. Do not add less than the indicated amounts.

The foregoing steps constitute the preliminary treatment of a sample for the destruction of nitrites, sulfides, etc., and for the oxidation of ferrous salts to the ferric condition.

The subsequent treatment is the same as when the Winkler method is used, starting with the shaking of the sample after the addition of the Winkler reagents. (See D, 2.1.) It should not be assumed that organic materials are absent when the permanganate treatment has been used.

2.2. Titration. As in the Winkler method (D, 2.3), a correction for the loss of dissolved oxygen by displacement with reagents should be applied to the amount measured out for titration. In this case, if the above amounts of reagents have been used, then the amount measured out for titration should be:

$$(200 \times 250) \div (250 - 5.7) = 204.7 \text{ ml.}$$

if it is desired to titrate 200 ml. of the original sample. (See Ref. F. S.

W. A. Com. Rep., p. 421, for the development of this figure).

## G. THE ALKALINE-HYPOCHLORITE MODIFICATION

The alkaline-hypochlorite modification should be used when the sample contains *sulfites, thiosulfates, polythionates, free chlorine or hypochlorites* (pulp and paper mill wastes, chlorinated effluents, etc.). This method is not a corrective for interfering materials in suspensions of river mud or sludge.

### 1. Reagents

1.1. Alkaline-hypochlorite solution. Two N NaOCl and 0.1 N NaOH. This solution may be prepared by passing chlorine gas through a 2.1 N NaOH solution, with cooling, until a 1 ml. test portion of the chlorinated solution requires about 20 ml. of 0.1 N thiosulfate for the neutralization of the iodine released upon acidification in the presence of an iodide.

This solution may be made by using commercial hypochlorite salts in place of pure sodium hypochlorite, after settling out the  $\text{CaCO}_3$  which is precipitated.

The alkaline-hypochlorite solution should be kept tightly stoppered when not in use and its strength should be checked at least every week.

1.2. Iodide solution. Approximately 1 N KI or NaI. Dissolve 17 g. of KI or 15 g. of NaI in distilled water and make up to 100 ml. This solution should be preserved by the addition of 1 ml. of 1 N NaOH to each 100 ml. of solution.

1.3. Sodium sulfite solution. 0.1 N. Add 6.3 g. of  $\text{Na}_2\text{SO}_3$  or 12.6 g.



of  $\text{Na}_2\text{SO}_3 \cdot 7\text{H}_2\text{O}$  to 1 liter of distilled water. This solution is rapidly oxidized by the air. Solutions more than a week old should not be used unless tests show that they have not lost more than 20 per cent in strength.

## 2. Procedure

Polythionates and similar compounds in pulp and paper mill wastes break down readily in alkaline solution to form sulfites and thiosulfates. The oxidation of such compounds must, therefore, be carried to the formation of sulfates before the addition of the alkaline-iodide solution.

A preliminary treatment, according to the Rideal-Stewart modification, is ineffective, as the oxidation of sulfites, thiosulfates and polythionates to sulfates is incomplete when permanganate is used. The error with samples containing 0.25 per cent of digestive waste from the manufacture of sulfite pulp may amount to 7 or 8 parts per million of dissolved oxygen, the results being too low.

With such samples a practical degree of accuracy may be secured through preliminary treatment with hypochlorite and iodine, as described below. At best, however, this procedure gives low results, the error with 0.25 per cent of digester waste amounting to about 1 ppm. Delays at any stage of the process should be avoided.

The procedure given below consists in a preliminary treatment of the sample with an alkaline solution of sodium hypochlorite for the decomposition of polythionates and for the oxidation of sulfites, thiosulfates, etc. to sulfates. The subsequent treat-

ment (see 2.1.3 below) calls for the removal of the undecomposed hypochlorite through the liberation of iodine, which is in turn removed with a dilute solution of sodium sulfite. Dissolved oxygen may then be determined by the regular Winkler procedure.

Beginning with paragraph 2.1.3 the procedure given below should also be used when dissolved oxygen is to be determined in samples containing free chlorine or hypochlorites. For the reason that tetrathionates are readily decomposed in alkaline solution, sodium thiosulfate should not be used for the removal of iodine liberated from iodides by free chlorine and its compounds.

A preliminary treatment with hypochlorite is effective in counteracting interference due to nitrites or ferrous salts, although for this purpose it is perhaps simpler to use the permanganate treatment.

### 2.1. Addition of reagents.

2.1.1. Add enough of the alkaline-hypochlorite reagent to oxidize the sample, avoiding a great excess.

With river water polluted with sulfite waste, 0.2 ml. of the alkaline-hypochlorite reagent may be added as a trial amount. In oxygen demand work, add 1 ml. of hypochlorite solution for each ml. of digester waste present in the bottle under examination.

2.1.2. Mix well by inverting rapidly a few times. The duration of this treatment should not greatly exceed 20 seconds.

If the period of contact with the alkaline-hypochlorite is unduly prolonged, low results will be obtained,

owing to the absorption of dissolved oxygen by the decomposition products of the organic matter.

2.1.3 Acidify the sample by adding 1 ml. of 3.6 N  $\text{H}_2\text{SO}_4$  (10 per cent solution by volume) and add 1 ml. of N KI (or NaI) to release iodine. Shake.

2.1.4. Neutralize the liberated iodine with 0.1 N  $\text{Na}_2\text{SO}_3$ , using 0.2 ml. of starch solution as an inside indicator. If the end point is overstepped, the blue color should be restored with 0.1 ml. portions of potassium bi-iodate solution of equivalent strength.

The reaction between sulfites and iodine to form sulfates is complete only in very dilute solution. Under the given experimental conditions the results will be relatively low when more than 3 ml. of 0.1 N sulfite are required for the neutralization of the iodine. On the other hand, if only 0.1 ml. or thereabouts of 0.1 N sulfite are required, it may be assumed that an insufficient amount of alkaline-hypochlorite has been used.

2.1.5. Add 1 ml. of manganous sulfate solution and 1.3 ml. of alkaline-iodide solution.

A slight excess of the alkaline-iodide reagent is used in order to neutralize the acid added during the preliminary treatment.

2.1.6. For the subsequent treatment see "The Winkler Method," D, 2.1, beginning with the shaking of the sample.

#### H. THE ALUM FLOCCULATION MODIFIED WINKLER PROCEDURE

This procedure should be used on *raw sewage samples, and samples con-*

*taining suspended solids* which interfere with the dissolved oxygen determination by any of the methods listed above. Suspensions and dilutions of sludge and river muds often indicate considerable so-called "immediate oxygen demand." This so-called "immediate demand" on such samples is in part an interference with the Winkler method and this can largely be corrected by removing the solids by alum flocculation before the Winkler procedure is applied.

#### 1. Reagents

1.1. Ten per cent alum solution. Dissolve 10 g. of potassium aluminum sulfate in 90 ml. of distilled water.

1.2. Concentrated ammonium hydroxide solution (28 per cent ammonia).

#### 2. Procedure

The procedure for the alum flocculation modification is outlined under Part II, Sec. 10, J, 5.1-5.3, page 135.

#### I. DISSOLVED OXYGEN IN ACTIVATED SLUDGE MIXED LIQUOR

Dissolved oxygen, so very important in activated sludge plant control, cannot be determined on settled mixed liquor because the rapid utilization of oxygen by the sludge removes a large percentage of it from solution during settling. To stop the oxygen utilization, hasten coagulation and settling and also destroy any nitrites that may be present, an inhibiting reagent is used. While any one chemical in this reagent is somewhat effective, the recommended combination is the most effective.

## 1. Reagents

1.1. Special inhibiting solution containing sulfamic acid, acetic acid and copper sulfate. For this reagent two solutions, one containing 32 grams of sulfamic acid ( $\text{NH}_2\text{SO}_2\text{OH}$ ) in 475 ml. of distilled water, and one containing 50 grams of copper sulfate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) in 500 ml. of water, are prepared. Mix these solutions and add 25 ml. of glacial acetic acid to complete the reagent.

Solution of the sulfamic acid may be accomplished by stirring, but heat should not be used to facilitate solution, nor should the mixture be exposed to heat at any subsequent time as this hastens the hydrolysis of the acid. The technical grade sulfamic acid may be used; any turbidity arising from this source may be removed by filtration or disregarded as it does not affect the efficiency of the reagent.

## 2. Procedure

2.1. Collection of samples. Add 10 ml. of the inhibiting reagent to a quart sampling bottle and lower carefully into the tank until the bottle is full. Then withdraw slowly, making sure that the bottle is full to overflowing. If different sample volumes are taken, 1 ml. of inhibiting reagent should be used per 100 ml. of sample.

Insert stopper forcing out some liquid to avoid trapping air, and shake carefully to mix inhibiting reagent more thoroughly with aeration mixture.

Allow sludge to settle and siphon out 250 ml. into a glass stoppered dissolved oxygen bottle.

2.2. Determination of dissolved oxygen. Determine the dissolved oxy-

gen by the sodium azide modification of the Winkler technic (E, page 129). For plant control the iodine color may be compared colorimetrically with color standards made up with  $\text{K}_2\text{Cr}_2\text{O}_7$  and  $\text{CoCl}_2$  solutions.

## J. OUTLINE FOR DISSOLVED OXYGEN PROCEDURE

-For convenience of reference, the following outline has been prepared by condensing the detailed instructions given above.

### 1. The Winkler Method

1.1. When a preliminary treatment is unnecessary the test is started by adding 1 ml. each of the  $\text{MnSO}_4$  soln. and alkaline-iodide solutions. Lesser amounts may be added under certain conditions.

1.2. In the practical absence of organic matter it is permissible to shake each bottle vigorously, passing from one bottle to another of a series. A second shaking is advisable. In the presence of much organic matter, treat each sample individually as directed in detailed instructions.

1.3. Acidify with 1 ml. concd.  $\text{H}_2\text{SO}_4$ .

1.4. Distribute the liberated iodine uniformly throughout the bottle; measure out a sample and titrate with 0.025 N  $\text{Na}_2\text{S}_2\text{O}_3$ , using starch as an indicator as described in J, 7.1-7.5, page 138.

### 2. The Sodium Azide Modification

2.1. Procedure is identical with that of the Winkler method except that the alkaline-iodide-sodium azide solution is substituted for alkaline-iodide solution, and 1.5 ml. of concd.



$\text{H}_2\text{SO}_4$  are recommended instead of 1 ml. for acidification.

2.2. The alternate preliminary azide procedure consists of treating the sample with 0.7 ml. of concd.  $\text{H}_2\text{SO}_4$  and 1.0 ml. of 2.0 per cent  $\text{NaN}_3$  solution and after 10 minutes contact continuing as in 3.5 and 3.6, below.

### 3. *Preliminary Treatment with Permanganate and Fluoride*

3.1. Add 0.7 ml. of concd.  $\text{H}_2\text{SO}_4$ .

3.2. Add enough  $\text{KMnO}_4$  soln. to obtain a violet tinge. Add 2 ml. of fluoride solution if appreciable amounts of iron salts are present.

3.3. Allow to stand until the reaction of the permanganate with readily oxidizable substances is judged complete (5 minutes or less).

3.4. Decolorize with the minimum possible amount of oxalate solution. The decolorization must proceed in the dark when iron salts are present.

3.5. When a preliminary treatment with permanganate has been applied, add 1 ml. of  $\text{MnSO}_4$  soln. and 3 ml. of alkaline-iodide reagent.

3.6. The subsequent treatment of the sample is the same as when the regular Winkler method is used (J, 1.3, 1.4, page 135).

### 4. *Preliminary Treatment with Alkaline-Hypochlorite*

4.1. Add a suitable amount of alkaline-hypochlorite reagent.

4.2. Shake for 20 seconds.

4.3. Add 1 ml. each of 3.6 N  $\text{H}_2\text{SO}_4$  (10 per cent solution by volume) and 1 N KI. Shake.

4.4. Add 0.2 ml. of starch solution and neutralize the iodine with 0.1 N

sulfite solution, restoring the blue color with 0.1 ml. portions of bi-iodate solution of equivalent strength.

4.5. When the alkaline-hypochlorite modification has been used, add 1 ml. of  $\text{MnSO}_4$  soln. and 1.3 ml. of alkaline-iodide.

4.6. The subsequent treatment of the sample is the same as when the regular Winkler method is used (J, 1.3, 1.4, page 135).

### 5. *Alum Flocculation for Sewage Sludges and Muds*

5.1. Collect 500 to 1000 ml. samples or dilutions in glass stoppered bottles, using the usual precautions against aeration of samples.

5.2. Add 10 ml. of 10 per cent alum solution followed by 1 or 2 ml. of concd.  $\text{NH}_4\text{OH}$ . Stopper and whirl for about 1 minute.

5.3. After settling the "floc" about 10 minutes, siphon the clear supernatant liquid into D.O. bottles and proceed with the Winkler method (J, 1.1-1.4, page 135).

### 6. *Dissolved Oxygen in Activated Sludge Mixed Liquor*

6.1. Add 10 ml. of inhibiting solution to 1 quart sample collecting bottle and collect sample carefully.

6.2. Insert stopper, mix thoroughly and settle.

6.3. Siphon clear liquor into a 250 ml. glass stoppered dissolved oxygen bottle.

6.4. Determine the dissolved oxygen by the azide modification (J, 2) or colorimetrically for plant operation purposes.

TABLE 14.—SOLUBILITY OF OXYGEN IN FRESH WATER AND IN SEA WATER OF STATED DEGREES OF SALINITY AT VARIOUS TEMPERATURES WHEN EXPOSED TO AN ATMOSPHERE CONTAINING 20.9 PER CENT OF OXYGEN UNDER A PRESSURE OF 760 mm.\*

(Calculated by G. C. Whipple and M. C. Whipple from measurements of C. J. J. Fox.)

° C.	Chlorides in sea water (parts per million)					Difference per 100 ppm. Cl	Dissolved oxygen in chloride-free water	
	0	5000	10000	15000	20000			
° C.	Dissolved oxygen in parts per million by weight					ppm.	° C.	ppm.
0	14.62	13.79	12.97	12.14	11.32	0.0165	30	7.6
1	14.23	13.41	12.61	11.82	11.03	.0160	31	7.5
2	13.84	13.05	12.28	11.52	10.76	.0154	32	7.4
3	13.48	12.72	11.98	11.24	10.50	.0149	33	7.3
4	13.13	12.41	11.69	10.97	10.25	.0144	34	7.2
5	12.80	12.09	11.39	10.70	10.01	.0140	35	7.1
6	12.48	11.79	11.12	10.45	9.78	.0135	36	7.0
7	12.17	11.51	10.85	10.21	9.57	.0130	37	6.9
8	11.87	11.24	10.61	9.98	9.36	.0125	38	6.8
9	11.59	10.97	10.36	9.76	9.17	.0121	39	6.7
10	11.33	10.73	10.13	9.55	8.98	.0118	40	6.6
11	11.08	10.49	9.92	9.35	8.80	.0114	41	6.5
12	10.83	10.28	9.72	9.17	8.62	.0110	42	6.4
13	10.60	10.05	9.52	8.98	8.46	.0107	43	6.3
14	10.37	9.85	9.32	8.80	8.30	.0104	44	6.2
15	10.15	9.65	9.14	8.63	8.14	.0100	45	6.1
16	9.95	9.46	8.96	8.47	7.99	.0098	46	6.0
17	9.74	9.26	8.78	8.30	7.84	.0095	47	5.9
18	9.54	9.07	8.62	8.15	7.70	.0092	48	5.8
19	9.35	8.89	8.45	8.00	7.56	.0089	49	5.7
20	9.17	8.73	8.30	7.86	7.42	.0088	50	5.6
21	8.99	8.57	8.14	7.71	7.28	.0086		
22	8.83	8.42	7.99	7.57	7.14	.0084		
23	8.68	8.27	7.85	7.43	7.00	.0083		
24	8.53	8.12	7.71	7.30	6.87	.0083		
25	8.38	7.96	7.56	7.15	6.74	.0082		
26	8.22	7.81	7.42	7.02	6.61	.0080		
27	8.07	7.67	7.28	6.88	6.49	.0079		
28	7.92	7.53	7.14	6.75	6.37	.0078		
29	7.77	7.39	7.00	6.62	6.25	.0076		
30	7.63	7.25	6.86	6.49	6.13	.0075		

\* Under any other barometric pressure, P, the solubility may be obtained from the corresponding value in the table by the formula:

$$S' = S \frac{P}{760} = S \frac{P'}{29.92} \text{ in which } S' = \text{Solubility at } P \text{ or } P',$$

S = Solubility at 760 mm. or 29.92 inches.  
P = Barometric pressure in mm. and  
P' = Barometric pressure in inches.

The second decimal place in the above table is not accurately known. The average difference from the mean of five different investigators represents 0.07 ppm. Until further data are obtained, however, the second decimal place has been retained in the table.

## 7. Titration

7.1. In general, the titration should be commenced within one hour after the final acidification. (See detailed instructions for tolerance in special cases).

7.2. For the titration it is convenient to pour out a portion of the iodine solution which corresponds to 100 or 200 ml. of the original sample (D, 2.3 and F, 2.2, pages 128 and 132).

7.3. Titrate to a pale straw color with 0.025 N thiosulfate.

7.4. Add 1 or 2 ml. of starch solution and titrate rapidly to the first disappearance of the blue color, neglecting subsequent recolorations.

7.5. The titration should not be delayed, once the sample has been poured.

## 8. Calculation of Results

8.1. Dissolved oxygen shall be reported in parts per million by weight and may be calculated as follows:

ppm. dissolved oxygen =  $[200 \times \text{ml. of } 0.025 \text{ N Na}_2\text{S}_2\text{O}_3] \div \text{ml. of sample titration}$  (see Sec. 10, D, 2.3 and F, 2.2).

8.2. If the results are desired in ml. of oxygen gas at 0° C. and 760 mm. pressure, the ppm. of D.O. are to be multiplied by 0.698.

8.3. To express the results as per cent of saturation at 760 mm. atmospheric pressure, the solubility data in Table 14 may be used. Formulae for correcting the solubilities to barometric pressures other than mean sea level are given below the table. These corrections are satisfactory for elevations below 2500 ft. and for temperatures below 25° C. At higher elevations and temperatures, the barometric

pressure must be corrected for the aqueous vapor pressure.

8.4. The solubility of dissolved oxygen in distilled water at any barometric pressure  $P$ , temperature  $t^\circ\text{C.}$ , and the saturated vapor pressure  $u$  for the given  $t^\circ\text{C.}$ , may be calculated between the temperatures of 0 and 30° C. by Eq. (1) and between the temperatures 30 and 50° C. by Eq. (2).

ppm. of D.O. at 0–30° C. =  $[0.678 (P - u)] \div 35 + t^\circ\text{C.}$  Eq. 1.

ppm. of D.O. at 30–50° C. =  $[0.827 (P - u)] \div 49 + t^\circ\text{C.}$  Eq. 2.

The solubility data for temperatures from 30 to 50° C. in the last column of Table 14 were calculated by Eq. (2).

## BIBLIOGRAPHY

- WINKLER, L. W. The determination of dissolved oxygen in water. *Ber.*, 21, 2843 (1888).
- RIDEAL, S., AND STEWART, G. G. The determination of dissolved oxygen in waters in the presence of nitrites and of organic matter. *Analyst*, 26, 141 (1901).
- FOX, C. J. J. On the coefficients of absorption of nitrogen and oxygen in distilled water and sea water and atmospheric carbonic acid in sea water. *Trans. Faraday Soc.*, 5, 68 (1909).
- WHIPPLE, G. C., AND WHIPPLE, M. C. Solubility of oxygen in sea water. *J. Am. Chem. Soc.*, 33, 362 (1911).
- GREENFIELD, R. E., AND MICKLE, F. L. A new sampler for collecting dissolved oxygen samples. *Ill. State Water Survey Bull.*, 16, 197 (1920).
- BUSWELL, A. M., AND GALLAGHER, W. W. The determination of dissolved oxygen in the presence of iron salts. *Ind. Eng. Chem.*, 15, 1186 (1923).
- ALSTERBERG, G. Methods for the determination of elementary oxygen dissolved in water in the presence of nitrite. *Biochem. Z.*, 159, 36 (1925).
- THERIAULT, E. J. The determination of dissolved oxygen by the Winkler method. *Pub. Health Bull. No. 151* (1925), Supt. of Documents, Washington.



- NICHOLS, M. S. Stabilized starch indicator. *Ind. Eng. Chem., Anal. Ed.*, 1, 215 (1929).
- THERIAULT, E. J., AND McNAMEE, P. D. Dissolved oxygen in the presence of organic matter, hypochlorites and sulfite wastes. *Ind. Eng. Chem., Anal. Ed.*, 4, 59 (1932).
- FEDERATION OF SEWAGE WORKS ASSNS. Committee Report. The determination of dissolved oxygen by the Winkler method. *Sew. Works J.*, 3, 413 (1932).
- MOORE, W. ALLAN. The solubility of atmospheric oxygen in sewage. *Sew. Works J.*, 10, 241 (1938).
- RUCHHOFT, C. C., MOORE, W. ALLAN, AND PLACAK, O. R. Determination of dissolved oxygen by the Rideal-Stewart and Alsterberg modifications of the Winkler method. *Ind. Eng. Chem., Anal. Ed.*, 10, 701 (1938).
- BARNETT, G. R., AND HURWITZ, E. The use of sodium azide in the Winkler method for the determination of dissolved oxygen. *Sew. Works J.*, 11, 781 (1939).
- RUCHHOFT, C. C., AND MOORE, W. ALLAN. The determination of biochemical oxygen demand and dissolved oxygen of river mud suspensions. *Ind. Eng. Chem., Anal. Ed.*, 12, 711 (1940).
- PLACAK, O. R., AND RUCHHOFT, C. C. Comparative study of the azide and Rideal-Stewart modifications of the Winkler method in the determination of biochemical oxygen demand. *Ind. Eng. Chem., Anal. Ed.*, 13, 12 (1941).
- RUCHHOFT, C. C., AND PLACAK, O. R. Studies of sewage purification. XVI. Determination of dissolved oxygen in activated sludge sewage mixtures. *Sew. Works J.*, 14, 638 (1942).

## 11. Biochemical Oxygen Demand

### A. DILUTION METHOD FOR SEWAGE

The biochemical oxygen demand (frequently referred to as B.O.D.) of sewage, sewage effluents, polluted waters or industrial wastes is the oxygen (in parts per million) required during stabilization of the decomposable organic matter by aerobic bacterial action. Complete stabilization requires more than 100 days at 20° C., but such long periods of incu-

bation are impracticable in any but research investigations. Consequently a much shorter period of incubation is used. Incubation for 1, 2, 5, 10 or 20 days at 20° C. is customary, and the 5 day period is recommended as the standard procedure. Conversion of the data from one incubation period to another, or from one temperature to another, may be approximated.

It is essential that normal growth of bacteria and plankton be permitted in the diluted samples; consequently reliable results cannot be obtained if the diluted sample contains caustic alkalinity, acid, free chlorine or any other bactericidal substance.

In such cases the bactericidal substance must be neutralized, reduced or removed if possible, *and the sample, or diluting water, seeded with normal sewage organisms.* Filtered sewage should not be used for seeding because protozoa or other higher micro-organisms necessary for a completely satisfactory flora are absent.

In order to obtain comparable results from two or more dilutions, the dilution water must be free from an appreciable oxygen demand of its own, and also must possess no germicidal properties from residual chlorine, caustic alkalinity, or copper from the lining of the still.

A satisfactory water should have a depletion of less than 0.2 ppm. of dissolved oxygen in 5 days incubation at 20° C., and should not be bactericidal. These requirements have led to the use of a synthetic dilution water. Distilled water alone and chemically treated tap waters should not be used. Chloramine treated tap waters should be passed through charcoal filters be-

fore distillation, to remove the chloramine compounds which are volatile.

### 1. Apparatus

1.1. Incubation bottles should be of 250 to 300 ml. capacity with ground glass stoppers, cleansed with chromic acid mixture, carefully rinsed and drained thoroughly before use. As a precaution against drawing air into the dilution bottle during incubation a water seal is recommended.

1.2. An air incubator or water bath thermostatically controlled at  $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$ . should be used.

### 2. Reagents

2.1. Standard dilution water. The standard dilution water is prepared by adding small quantities of four solutions to good quality distilled water.

There are four stock solutions to be prepared by dissolving the cp. reagent quality salts in liter quantities of distilled water, as follow:

(a) Ferric chloride 0.25 g.  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ .

(b) Calcium chloride 11.0 g.  $\text{CaCl}_2$  (anhyd.).

(c) Magnesium sulfate 10.0 g.  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .

(d) Phosphate buffer stock solution. Dissolve 34 g. of potassium acid phosphate ( $\text{KH}_2\text{PO}_4$ ) in 500 ml. of distilled water. Add approximately 175 ml. of 1 N NaOH (more or less) until a pH of 7.2 is reached. Then add 1.5 g.  $(\text{NH}_4)_2\text{SO}_4$  and dilute to 1 liter.

The distilled water used for these solutions and dilution water must be of good quality, containing less than

0.01 ppm. copper,\* and aerated to remove excess  $\text{CO}_2$  and to saturate with  $\text{O}_2$ .

The standard dilution water is prepared by adding to each liter of distilled water 0.5 ml. of soln. (a), 2.5 ml. of (b), 2.5 ml. of (c), and 1.25 ml. of (d).

In some cases it is desirable to use the river water, into which the effluent or waste flows, as a dilution water for the B.O.D. determination. Tests using standard dilution water, however, should be run for comparison.

### 3. Procedure

3.1. For unchlorinated sewages and effluents.

3.1.1. pH adjustment. If the sample contains caustic alkalinity or acid, neutralize it to about pH 7.0 with dilute acid or sodium carbonate, using brom thymol blue indicator and seed with sewage organisms as described in 3.1.2.

3.1.2. Seeding. Seeding of sterile industrial wastes or sewages is to be accomplished by adding settled sewage or polluted river water to the dilution water in amounts to give about 0.5 ppm. *depletion in the blank*. In case of chlorinated sewages or effluents the seeding may not be effective unless all the chlorine and chloramine compounds have been dissipated (A, 3.2, page 142).

3.1.3. Supersaturation. Samples collected during the winter months or from localities where algae are actively growing may be supersaturated with dissolved oxygen (over 9.17 parts

\* Most commercial distilled waters contain appreciable amounts of copper. Testing such waters for very small quantities of copper is essential.

per million with reference to a temperature of 20° C.).

To prevent the loss of supersaturated oxygen during incubation, the following procedures should be applied whenever the dissolved oxygen content exceeds 9.0 parts per million. The sample in a partly filled bottle should be brought to about 20° C. and agitated vigorously. Application of suction in this operation will hasten removal of the excess gas. The sample may be diluted with deaerated water.

3.1.4. Dilution technic. Make several dilutions of the prepared sample in order to cover the range of depletions expected (usually 0.1 to 1.0 per cent of strong trade wastes; 1 to 5 per cent of normal and settled sewages; 5 to 25 per cent of oxidized effluents; and 25 to 100 per cent on polluted river waters).

This dilution may be accomplished as follows: Carefully siphon standard dilution water into a 500 or 1000 ml. graduated cylinder, half filling the cylinder without entrainment of air. Add the quantity of carefully mixed (not aerated by vigorous shaking) sample to make the desired dilution and fill to the 500 or 1000 ml. mark with dilution water.

Mix well with a plunger type mixing rod, avoiding the entrainment of air. Siphon the mixed dilution into incubation bottles (one for incubation and one for determination of the dissolved oxygen in the mixture, if desired), tightly stopper and fill the water seal.

Prepare succeeding dilutions of less concentration in the above manner or

by adding dilution water to the unused portion of the preceding dilution.

The dilution technic is greatly simplified when suitable amounts of sample are added directly to bottles of known capacity, and the bottle filled into the neck with just sufficient dilution water so that the stopper can be inserted without leaving an air bubble. Dilutions greater than 1 to 100 (1 per cent) may be made by diluting the waste in a volumetric flask before it is added to the incubation bottles for final dilution.

Fill two bottles completely with dilution water, stopper and fill the seal of one for incubation. The other bottle is for determining the dissolved oxygen of the dilution water before incubation, as a check on the quality of the dilution water. The 5 day oxygen consumption of unseeded dilution water should not exceed 0.2 parts per million.

Determine the dissolved oxygen on the undiluted sample. In practice this determination on crude sewages and settled effluents is not usually necessary, but it should be made on oxidized effluents.

Incubate the blank and the diluted samples for 5 days at 20° C., and then determine the dissolved oxygen in the incubated samples and the blank, using the sodium azide modification of the Winkler method (Part II, Sec. 10, E, page 129). (In special cases some of the other modifications may be necessary.) Those dilutions showing 40 to 70 per cent depletion of the initial oxygen content shall be considered most reliable.

3.1.5. Calculation.



Let  $p$  = per cent of dilution water expressed as a decimal.

$P$  = per cent of sample expressed as a decimal.

D.O. = ppm. dissolved oxygen as titrated.

D.O.<sub>b</sub> = ppm. D.O. in the incubated blank.

D.O.<sub>1</sub> = ppm. D.O. in the diluted sample after incubation.

D.O.<sub>s</sub> = ppm. D.O. in the original undiluted sample.

Then D.O.<sub>c</sub> = calculated D. O. available in dilution =  $(D.O._b \times p) + (D.O._s \times P)$ .

And ppm. B.O.D. =  $(D.O._c - D.O._1) \times (1/P)$ , or simplified without calculating the available D.O. then ppm. B.O.D. =  $(D.O._b - D.O._1) \times (1/P) - (D.O._b - D.O._s)$ .

No correction is made for the small loss or gain of dissolved oxygen in the dilution water during incubation because the calculation is based on the incubated blank and not on the dilution water before incubation.

If it is desired to determine the immediate oxygen demand of the sample the D.O. of the diluted sample should be run on an additional diluted sample within 15 minutes after making the dilution and this quantity subtracted from the calculated initial D.O. of the dilution. This initial D.O. is calculated as indicated above except that the initial D.O. of the dilution water before incubation is used in place of that of the incubated blank. Calculation:

ppm. immediate oxygen demand =  $(D.O._c - D.O._1 \text{ of diln. after 15 min.}) \times (1/P)$ .

3.2. For chlorinated sewage and effluents.

3.2.1. If acid to phenolphthalein. To a portion of a sample of 100 to 500 ml. depending on the chlorine residual present, add 10 ml. of 20 per cent potassium iodide (KI) solution and titrate the liberated iodine with 0.025 N sodium acid sulfite ( $\text{NaHSO}_3$ ) solution which has been standardized against 0.025 N iodine solution immediately before using. The presence of nitrite will produce a false test for free chlorine.

To a suitable volume, for example, 1 liter of sample, add just sufficient 0.025 N  $\text{NaHSO}_3$  solution to dechlorinate, as calculated from the above titration. After mixing, test 10 ml. with ortho-tolidine solution to check absence of free chlorine.

To another 10 ml. portion add 1 drop of 0.01 N iodine solution and a few drops of starch solution; a blue color should develop, indicating no appreciable excess of  $\text{NaHSO}_3$ .

Proceed with the B.O.D. dilutions, determining the D.O. of the dechlorinated sample and seeding the dilution water with unchlorinated settled sewage or river water to give depletions in blank of 0.5 ml. of 0.025 N sodium thiosulfate (0.5 ppm.).

3.2.2. If alkaline to phenolphthalein. To 100 ml. of the sample add a few drops of brom thymol blue indicator and titrate with 0.02 N  $\text{H}_2\text{SO}_4$  to the green neutral color (pH 7.0).

To another portion of the sample, 100 ml. to 500 ml., depending on the chlorine residual present, add the volume of 0.02 N  $\text{H}_2\text{SO}_4$  required (see paragraph above), then add 10 ml. of 10 per cent potassium iodide solution and titrate with 0.025 N  $\text{NaHSO}_3$  solution.

To a suitable volume, for example, 1 liter of sample, add 0.02 N  $\text{H}_2\text{SO}_4$  and  $\text{NaHSO}_3$  as calculated from the above titrations, mix, test for free chlorine and for excess sodium acid sulfite and proceed with the B.O.D. dilutions as in A, 3.2.1.

In the final B.O.D. calculation, correction is made for the addition of sodium acid sulfite and sulfuric acid solutions if added.

3.2.3. For small residual (less than 0.5 ppm.). To a suitable volume of sample, for example, 1 liter, add 0.025 N  $\text{NaHSO}_3$  as calculated from the chlorine residual (determined by the ortho-tolidine test, noting the absence of nitrite by making the usual test), Part II, Sec. 18, page 147.

Thus if the residual chlorine is 0.33 ppm., add to 1 liter 0.37 ml. of 0.025 N  $\text{NaHSO}_3$ . After ten minutes, test 1 ml. in a spot plate with three drops of ortho-tolidine to note the absence of chlorine.

It may also be expected that residuals of a few tenths of a part per million will often disappear on standing an hour or two, or if the residual is low it will disappear when the sewage is diluted with B.O.D. dilution water, making the addition of  $\text{NaHSO}_3$  unnecessary. *Proper seeding of dilution water must be used, however, even for low residuals when diluted.*

## B. DILUTION METHOD FOR SLUDGES AND MUDS

The precautions to be used in the determination of the dissolved oxygen of sewages, sludges and muds will be found in Part II, Sec. 10, A, page 124.

## 1. Procedure

The procedure for more accurate determinations of the biochemical oxygen demand on sludges and mud dilutions involves:

(1) The calculation of the initial dissolved oxygen of the dilution,

(2) The maintenance of the solids in suspension during the incubation period, and

(3) The determination of the dissolved oxygen upon incubated samples after clarification by alum flocculation (Part II, Sec. 10, H, page 134).

## BIBLIOGRAPHY

- MOHLMAN, F. W., EDWARDS, G. P., AND SWOPE, GLADYS. Technic and significance of the biochemical oxygen demand determination. *Ind. Eng. Chem.*, 20, 242 (1928).
- PHELPS, E. B. Stream pollution from the operators' point of view. *Sew. Works J.*, 2, 555 (1930).
- BUTTERFIELD, C. T., PURDY, W. D., AND THERIAULT, E. J. The influence of plankton on the biochemical oxidation of organic matter. *Pub. Health Repts.*, 46, 494 (1931).
- THERIAULT, E. J. Detailed instructions for the performance of the dissolved oxygen and biochemical oxygen demand tests. *Pub. Health Reprints, Suppl. 90* (1931).
- THERIAULT, E. J., MCNAMEE, P. D., AND BUTTERFIELD, C. T. Experimental studies of natural purification in polluted waters. V. Selection of dilution water for use in oxygen demand tests. *Pub. Health Repts.*, 46, 1084 (1931).
- Sixth Annual Report "Ohio Conference on Sewage Treatment," page 64 (1932).
- HOSKINS, J. K. The oxygen demand test and its applicability. *Sew. Works J.*, 5, 923 (1933).
- LEA, WILLIAM L., AND NICHOLS, M. STARR. Influence of substrate on biochemical oxygen demand. *Sew. Works J.*, 8, 435, (1936).
- LEA, WILLIAM L., AND NICHOLS, M. STARR. Influence of phosphorous and nitrogen on biochemical oxygen demand. *Sew. Works J.*, 9, 34 (1937).

NICHOLS, M. STARR. Uses and abuses of biochemical oxygen demand. *Am. J. Pub. Health*, 29, 901 (1939).

RUCHHOFT, C. C., AND MOORE, W. ALLAN. Determination of biochemical oxygen demand in river mud suspensions. *Ind. Eng. Chem., Anal. Ed.*, 12, 711 (1940).

RUCHHOFT, C. C. Report on the cooperative study of dilution waters made for the Standard Methods Committee of the Federation of Sewage Works Associations. *Sew. Works J.*, 13, 669 (1941).

## 12. Relative Stability of Effluents

Relative stability may be defined as the per cent ratio of oxygen available as dissolved oxygen, nitrite and nitrate oxygen to the total oxygen required to satisfy the biochemical oxygen demand, Part II, Sec. 11, page 139. This percentage or ratio may be approximately indicated by determining the number of days required to exhaust the available oxygen in the sample, using methylene blue as an indicator.

The use of this determination is rapidly decreasing, as it is being replaced by the more exact determinations of dissolved oxygen, nitrite, nitrate and biochemical oxygen demand. For small plants with limited laboratory facilities, however, the stability toward methylene blue fulfils a very useful service in indicating the satisfactory operation of the biological oxidation processes.

Effluents which contain caustic alkalinity or acidity should be neutralized to brom thymol blue and seeded with sewage bacteria before adding the indicator. The method is inapplicable if bactericidal substances that cannot be removed are present.

The theoretical relation between the time required for decolorization of methylene blue at 20° C. and the rela-

tive stability percentage is given in Table 15, and is represented by the relation  $S = 100 (1 - 0.794^t)$  in which S is the stability in per cent and t is the time in days required for decolorization at 20° C.

TABLE 15.—RELATIVE STABILITY NUMBERS

Time required for decolorization at 20° C.	Relative stability S.	Time required for decolorization at 20° C.	Relative stability S.
Days	Percentage	Days	Percentage
0.5	11	8.0	84
1.0	21	9.0	87
1.5	30	10.0	90
2.0	37	11.0	92
2.5	44	12.0	94
3.0	50	13.0	95
4.0	60	14.0	96
5.0	68	16.0	97
6.0	75	18.0	98
7.0	80	20.0	99

### 1. Reagent

1.1. Methylene blue.. Dissolve 0.5 g. of U.S.P. methylene blue in distilled water and make up to 1 liter.

### 2. Procedure

Fill a 150 ml. glass stoppered bottle with sample, avoiding aeration. Add exactly 0.4 ml. of methylene blue indicator solution below the surface of the liquid and mix by inverting the bottle. Incubate at 20° C. with a water seal, observing the samples daily until decolorization takes place.

Report the days required for decolorization, or if preferred, the relative stability percentage shown in Table 15. If other sized incubation bottles are used a proportional amount of the indicator solution must be used.



### 13. Residue or Solids

#### A. TOTAL SOLIDS ON EVAPORATION

The results on total solids, volatile solids and fixed solids are subject to considerable error because of losses of volatile compounds during evaporation, of carbon dioxide and volatile minerals during ignition, and to the presence of calcium oxide in the ash. In the interpretation of results these possible errors must be recognized.

##### 1. Procedure for Total Solids

Evaporate 100 ml. of sample in an ignited and tared dish, dry to constant weight at 103° C., cool in desiccator and weigh. Drying for one hour at 103° C. is usually sufficient. The mg. per liter shall be reported as parts per million total solids.

$$\text{ppm. total solids} = \text{mg. of residue} \times 1000 \div \text{ml. of sample.}$$

##### 2. Procedure for Total Volatile and Fixed Solids

Total volatile and fixed solids are determined by igniting the above total solids at 600° C. in an electric muffle to constant weight (usually requiring 10 to 15 minutes). The mg. loss on ignition per liter shall be reported as parts per million volatile solids and the mg. residue per liter as parts per million fixed solids. Calculate as above.

#### B. SUSPENDED SOLIDS

##### 1. Reagents and Apparatus

1.1. Asbestos cream. Prepare a cream with distilled water and acid-washed, medium fiber asbestos, which is prepared particularly for Gooch crucible determinations. 2.5 to 5 g.

of asbestos in 900 ml. of water are satisfactory.

1.2. Gooch crucible and mat. Prepare a mat of asbestos fiber, from 3 to 5 mm. thick, in a Gooch crucible by gentle suction. Wash with 100 ml. of distilled water, dry at 103° C., cool and weigh. If volatile matter is to be determined by ignition the crucible and mat must be ignited, cooled and weighed.

##### 2. Procedure for Total Suspended Solids

Filter from 50 to 100 ml. of sample through the weighed Gooch crucible using suction. Wash with distilled water, dry at 103° C. for 1 hour, cool in a desiccator and weigh.

For the rapid determination of suspended solids, particularly of mixed liquor in activated sludge plants, the aluminum dish method (Part II, Sec. 25, page 157) should be used.

Report the mg. solids per liter as parts per million suspended solids. For samples of high suspended solids the original sample may be diluted and an aliquot portion analyzed.

##### 3. Procedure for Volatile and Fixed Suspended Solids

The volatilization of organic matter, carbonates and ammonium salts from sewage solids is subject to a great many errors. It should be done in an electric muffle furnace at 600° C.

Ignite the suspended solids for 10–15 minutes. Cool in a desiccator and re-weigh.

Report the mg. lost on ignition per liter as parts per million of volatile suspended solids and the mg. ash per

liter remaining as parts per million fixed suspended solids.

### C. DISSOLVED SOLIDS

Dissolved solids may be obtained by difference between total solids and suspended solids. Dissolved solids may also be determined by evaporating a filtered sample in accordance with Part II, Sec. 13, A, 1, page 145.

### D. SETTLEABLE SOLIDS

#### 1. *By Volume*

Fill an Imhoff cone to the liter mark with a thoroughly mixed sample. Settle for 0.75 hour, gently stir the sides of the cone with a rod or by spinning, settle 0.25 hour longer and record the ml. of settleable solids in the cone.

#### 2. *By Weight*

This technique defines settleable solids as that matter in sewage which will not stay in suspension during the settling period either by virtue of settling to the bottom or floating to the top.

Determine the suspended solids (Part II, Sec. 13, B, 2, page 145) in a sample of the sewage under investigation. Pour a well mixed sewage sample into a glass vessel not less than 9 cm. in diameter, using a quantity of sample not less than 1 liter but sufficient to insure a depth of 20 cm. A glass vessel of greater diameter and larger volume of sample may be used.

Allow to stand quiescent for one hour (the use of the theoretical detention period of the plant settling units is permissible if desired).

Without disturbing the settled material or that which may be floating,

remove 250 ml. of the sample from the center of the container at a point half way between the surface of the settled sludge and the liquid surface.

Determine the suspended solids in ppm. in all or in an aliquot portion of this supernatant liquor in accordance with standard procedures. This value in ppm. is equivalent to the non-settling solids.

ppm. by weight of settleable solids = ppm. susp. sol. - ppm. non-settling solids.

### BIBLIOGRAPHY

- THERIAULT, E. J., AND WAGENHALS, H. H. Studies of representative sewage plants. *Pub. Health Bull.*, 132 (1923).
- SYMONS, GEORGE E., AND MOREY, B. The effect of drying time on the determination of solids in sewage and sewage sludges. *Sew. Works J.*, 13, 936 (1941).
- FISCHER, A. J., AND SYMONS, GEORGE E. The determination of settleable sewage solids by weight. *Water Works & Sew.*, 91, 37 (1944).

### 14. Acidity

#### 1. *Reagents*

- 1.1. Phenolphthalein indicator.
- 1.2. Sodium hydroxide. 0.02 N solution.

#### 2. *Procedure*

Titrate 50 ml. of the settled sample in a 50 ml. graduated cylinder (the use of the cylinder is to minimize the loss of  $\text{CO}_2$  during the titration and to improve the visibility of the end point), using a plunger type stirring rod, 4 drops of phenolphthalein indicator, and 0.02 N NaOH solution until the first permanent pink color appears. Express the total acidity as ppm.  $\text{CaCO}_3$  or as milliequivalents of acid per liter.

**Calculation:**

ppm. acidity as  $\text{CaCO}_3$  = ml. 0.02 N NaOH  $\times 20$ .

milliequiv. of acid per liter = ml. 0.02 N NaOH  $\times 0.4$ .

**15. Alkalinity**

The method for sewage analysis is the same as that used in water analysis, Part I, Sec. 13, page 31. The titration may be carried on to advantage in a glass cylinder as described for acidity. This improves the end point in turbid solutions.

**16. pH Value**

The pH value shall be determined by the glass electrode procedure described in Part I, Sec. 11, page 28.

For purposes of plant control or where extremely accurate results are not required, colorimetric methods may be used to determine the pH of sewages, sewage effluents, sludges, etc. The technique is described in Appendix I, Sec. 10, page 224.

**17. Chloride**

If the sample contains sulfides, acidify 50 ml. of sample with  $\text{H}_2\text{SO}_4$  and oxidize the sulfides by heating with hydrogen peroxide for a few minutes. Cool, neutralize with  $\text{NaHCO}_3$ , dilute to the original volume, and proceed as in Part I, Sec. 37, B, 2, page 74.

**18. Residual Chlorine**

In general, the determination of residual chlorine in sewage is similar to the determination in water, with a few modifications in detail of technic.

The determination of residual chlorine in water, by both the ortho-tolidine and starch-iodide methods is

discussed thoroughly in Part I, Sec. 49, A, B, pages 93 and 98. That section presents the many pitfalls of the tests and the precautions to be observed.

In sewage, the differentiation of free available chlorine from combined available chlorine (chloramine) is not made and no attempt is made to correct residual chlorine results for false readings caused by other strong oxidizing agents.

Permanent standards or commercial colorimeters are to be preferred to temporary standards. The technic for preparing either type of standard is given in Part I, Sec. 49, A, 2 and 3, page 94. Information on light sources is given in Part I, Sec. 49, A, 3.9, page 97.

Directions for making reagents common to both water and sewage technics are the same, but are repeated here for convenience. The concentration of acid in the ortho-tolidine reagent is such that it will produce a suitable pH in the sample under examination even if the alkalinity of the sample is 2000 ppm. Higher alkalinities will require the addition of more ortho-tolidine reagent than is specified.

**A. ORTHO-TOLIDINE METHOD****1. Reagents**

1.1. Ortho-tolidine reagent. Dissolve 1.35 g. of ortho-tolidine dihydrochloride in 500 ml. distilled water. Add this solution, with constant stirring, to 500 ml. of dil. HCl made by mixing 350 ml. distilled water and 150 ml. of concd. HCl (sp. gr. 1.18-1.19).



1.2. Storage of ortho-tolidine reagent. The ortho-tolidine solution should be: (a) stored in amber bottles or in the dark, (b) protected at all times from direct sunlight, (c) used no longer than six months, (d) kept from contact with rubber and (e) maintained at normal temperatures.

At temperatures less than 0° C., the ortho-tolidine will precipitate from solution and cannot be redissolved easily. The use of reagent from which part of the ortho-tolidine has precipitated may lead to errors due to a deficiency of ortho-tolidine.

1.3. Standard chlorine solution. Pass chlorine gas into distilled or tap water until the solution contains approximately 0.1 mg. of chlorine per ml. In the absence of chlorine gas dilute a commercially prepared solution of hypochlorite to give a solution of approximately 0.1 mg. of chlorine in 1 ml. of solution. Standardize the solution and dilute so that 1 ml. will contain 0.1 mg. of chlorine.

*Standardization.* To 150 ml. of distilled water add 1 to 2 g. of KI crystals and dissolve, add 50 ml. of chlorine solution, 1 ml. of glacial acetic acid and allow 5 minutes for liberation of iodine. Then titrate with 0.025 N  $\text{Na}_2\text{S}_2\text{O}_3$ , using starch solution in the usual way, until the blue color has disappeared.

One ml. of 0.025 N  $\text{Na}_2\text{S}_2\text{O}_3$  is equivalent to 0.886 mg. of chlorine. Therefore

$$\text{mg. of Cl}_2 \text{ per ml.} = \text{ml. of } 0.025 \text{ N } \text{Na}_2\text{S}_2\text{O}_3 \times 0.886 \div 50.$$

## 2. Procedure

Add a 10 ml. sample of chlorinated sewage that has had at least a ten

minute contact period (and has been warmed to 20° C. if the sample is cold) to a cell or test tube containing 1 ml. of ortho-tolidine solution.

Place the treated sample in the dark during color development. Maximum color develops in less than 5 minutes and fading occurs thereafter. Therefore to obtain the proper color value, the reading should be made within 5 minutes. *With some wastes maximum color is attained immediately and the reading must be made before fading occurs.*

Color comparison should be made with color standards containing 0.0, 0.1, 0.2, 0.4, 0.6, 0.8, and 1 mg. of chlorine per liter, in a turbidity compensating colorimeter.

Temporary standards may be made by diluting the standard chlorine solution with zero chlorine demand distilled water. One ml. of std. chlorine soln. diluted to 100 ml. is equivalent to 1 mg. per liter (1 ppm.) of chlorine. Temporary standards must be made up with each determination.

For permanent standards see Part I, Sec. 49, A, 3.5, page 96, but note that the amount of sample is but 10 ml. and depth of liquid viewed is not the same as in Table 12, and adjustments may have to be made accordingly.

## B. STARCH-IODIDE METHOD

When nitrite N is present in amounts greater than 2.0 ppm., the ortho-tolidine method should not be followed. It may also be desired to determine chlorine in the presence of manganic Mn by an alternate method. It is noted that interference of the manganese does not occur at a pH

above 4.0, of ferric iron above a pH of 3.9, nor do nitrites interfere at a pH above 4.2.

Accordingly, the neutral starch-iodide method (in which acidification is omitted) may be used to determine chlorine when either nitrite nitrogen or manganic Mn interferes.

### 1. Reagents

1.1. Potassium iodide solution (KI). Dissolve 75 g. cp. KI (free from iodine and iodate) in 1 liter freshly boiled and cooled distilled water. If difficulty is experienced with stability of the solution, the solid substance may be used directly, since it is very soluble.

1.2. 0.1 N sodium thiosulfate. Make 0.1 N sodium thiosulfate by dissolving at least 25 g. of the cp.  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  in 1 liter of freshly boiled distilled water, standardizing it against potassium dichromate (1.3 below) after at least two weeks' storage. Boiled water is used because bacterial action decomposes thiosulfate solutions. Trouble may be avoided by the addition of a few ml. of chloroform.

1.3. Standardization of 0.1 N sodium thiosulfate. To 80 ml. of distilled water add, with constant mixing, 1 ml. of concd.  $\text{H}_2\text{SO}_4$ , 10 ml. of 0.1 N  $\text{K}_2\text{Cr}_2\text{O}_7$  and 10 ml. of KI soln. (1.1). Allow to stand six minutes in subdued light at laboratory temperature and titrate with the thiosulfate solution being standardized.

1.4. 0.01 N sodium thiosulfate. Stability of 0.01 N thiosulfate is improved if it is prepared by diluting an aged 0.1 N solution with freshly boiled distilled water. Boiled water

is used because bacterial action decomposes 0.01 N thiosulfate. Trouble may be avoided by the addition of a few ml. of chloroform. For accurate work, this solution should be standardized daily, in accordance with the directions given in 1.3 above, using 0.01  $\text{K}_2\text{Cr}_2\text{O}_7$  soln. if desired. The use of an automatic burette, of a type in which rubber does not come in contact with the solution, is advisable.

1.5. Starch solution. To 5 g. starch (potato, arrowroot or soluble) in a mortar, add a little cold water and grind to a thin paste. Pour into 1 liter of boiling distilled water, stir and allow to settle overnight. Use the clear supernatant. The solution should be preserved with salicylic acid (1.25 g. per liter) or with zinc chloride (4 g. per liter).

### 2. Procedure

2.1. Volume of sample. The amount of sample to be taken for titration is governed by the concentration of chlorine in the sample. It is suggested that, for quantities of residual chlorine of 1.0 ppm. or less, 1 liter be titrated; for residual chlorine concentrations between 1 ppm. and 10 ppm., 500 ml.; for residual chlorine concentrations above 10 ppm., proportionately less of the sample should be used. It is preferable to use sufficient sample so that not more than 20 ml. of 0.01 N  $\text{Na}_2\text{S}_2\text{O}_3$  is required.

2.2. Preparation for titration. The accuracy of this determination increases as the temperature is lowered. If the sample has a temperature above 20° C. it should be chilled at least to that point. Cooling in ice

water, or if desired, in an ice-salt water mixture, to 1° C., will further increase the sensitivity of the determination.

2.3. Titration. Add 0.01 N thio-sulfate from a burette until the yellow color of the liberated iodine is almost discharged. When liter samples are titrated, add 5 ml. starch, reducing this amount with smaller samples. The amount of starch used should be sufficient to give a deep blue color. After the addition of starch, titrate carefully, but rapidly, to the end point. Long contact of iodine and starch produces a blue compound which is decolorized with difficulty.

#### 2.4. Calculation of results.

ppm.  $\text{Cl}_2 = \text{ml. } 0.01 \text{ N } \text{Na}_2\text{S}_2\text{O}_3 \times 0.3546 \times 1000 \div \text{ml. sample.}$

2.5. Colorimetric determination. With chlorine residuals of 0.1 part per million and less, color comparisons of unknowns and freshly prepared chlorine standards may be made, adding to both samples and standards in 100 ml. Nessler tubes, 1 ml. KI soln. and 0.5 ml. starch solution and reading after full color development. The sensitivity of this procedure will be increased by cooling all tubes to 5° C. before the reagents are added.

#### C. SPOT PLATE TEST FOR FIELD USE

A rapid spot plate technic offers simplicity and speed for plant control work and field use.

##### 1. Apparatus and Reagents

1.1. Spot plates containing several depressions.

1.2. Pipette bulb and large bore one ml. pipette.

1.3. Ortho-tolidine reagent (A, 1.1, page 147).

1.4. Solutions of permanent standards, adjusted in color intensities and hues to match temporary standards in spot plates.

#### 2. Procedure

Add 3 drops of ortho-tolidine reagent to a depression in the spot plate. Pipette, by means of the pipette bulb, one ml. of the sample under examination directly and rapidly into the spot containing the ortho-tolidine reagent, and stir with the tip of the pipette. The color will usually develop with 45 seconds to one minute and fade thereafter.

Compare the color developed with permanent standards pipetted into another spot plate. Temporary standards may be used. Several samples may be spotted at the same time. The minimum readable color is between 0.05 and 0.1 ppm.

#### BIBLIOGRAPHY

- LEA, C. Chemical control of sewage chlorination, the use and value of the ortho-tolidine test. *Soc. Chem. Ind.*, 52, 245T (1933).
- SYMONS, GEORGE E. A modification of the chlorine demand test and the ortho-tolidine test for residual chlorine. *Sew. Works J.*, 9, 569 (1937).
- A. W. W. A. COMMITTEE REPORT. Control of chlorination. *J. Amer. W. W. Assn.*, 35, 1315 (1943).

#### 19. Chlorine Demand

Chlorine demand of water or sewage is the difference between the amount of chlorine applied and the amount of residual chlorine remain-



ing at the end of the contact period. The smallest amount of residual chlorine considered at all significant is 0.1 ppm. The demand for any given sewage varies with the amount of chlorine applied, time of contact, and temperature. Therefore, it is imperative that all conditions be standardized. For this reason the following definition has been set up to standardize the conditions of the determination.

*Definition.* The chlorine demand (C.D.) of sewage shall be defined as the number of parts per million (ppm.) of chlorine required to be added as chlorine water (1 g. of  $\text{Cl}_2$  per liter) to produce a residual chlorine content of 0.1 ppm. after fifteen minutes contact of the chlorine with the sewage. The residual chlorine is to be determined by the technic in Part II, Sec. 18, A, B, or C, page 147, 148 or 150.

Inasmuch as chlorine is ordinarily used for sewage chlorination, the chlorine demand should be determined with chlorine water since the chlorine demand of sewage may vary if hypochlorites are used in place of chlorine water. For more convenient use in the field or when facilities are not available for standardizing chlorine water, commercial solutions of hypochlorites (Part I, Sec. 49, A, 2.2, page 95) may be used. In general, the results will be of the same order of accuracy as with chlorine water.

### 1. Reagents

1.1. Chlorine water. By passing chlorine gas through distilled water a solution containing slightly more than 1 g. of chlorine per liter is produced. Tap water may be used in

preference to distilled water, particularly if the reagent is to be used in connection with control work.

This solution is not stable and must be standardized each time it is used or made fresh daily. Standardization is to be accomplished according to the directions in Part II, Sec. 18, A, 1.3, page 148, using 5 ml. of the chlorine water instead of 50 ml. as recommended.

$$\text{mg. of } \text{Cl}_2 \text{ per ml.} = \text{ml. } 0.025 \text{ N } \text{Na}_2\text{S}_2\text{O}_3 \times 0.886 \div 5.$$

1.2. Ortho-tolidine solution. Part II, Sec. 18, A, 1.1, page 147.

### 2. Procedure

Measure out five 250 ml. portions of sewage to be tested. To the first of these samples add the standard chlorine water, 0.5 ml. at a time (equivalent to approximately 2 ppm. of chlorine) with gentle stirring until a spot plate test, using three drops of ortho-tolidine solution and 1 ml. of the sample gives a readable yellow color (approx. 0.1 ppm.). Call this the immediate chlorine demand (I.C.D.).

Using the I.C.D. as the basic dosage, add to the other 250 ml. portions this amount of chlorine water, plus increasing quantities of  $\text{Cl}_2$  water, using increments of 0.2 to 0.5 ml. above the I.C.D. (It may be generally expected that the I.C.D. is approximately 60 to 80 per cent of the total 15 minute chloride demand).

Gently stir all flasks while adding the chlorine water and allow to stand for exactly fifteen minutes. During the contact period the samples should be protected from strong daylight. At the end of 15 minutes determine

the residual chlorine in each flask, using the technic given in Part II, Sec. 18, A, 2, page 148. For sewage chlorination control and field work the spot plate technic (Part II, Sec. 18, C, page 150) offers many advantages.

#### Calculation:

ppm. C.D. =  $\frac{[\text{ml. chlorine soln} \times \text{mg. Cl}_2 \text{ per ml.} \times 1000]}{\text{ml. of sample treated.}}$

#### BIBLIOGRAPHY

- LEA, C. Chemical control of sewage chlorination, the use and value of the ortho-tolidine test. *Soc. Chem. Ind.*, 52, 245 T (1933).
- SYMONS, G. E. A modification of the chlorine demand test and the ortho-tolidine test for residual chlorine. *Sew. Works J.*, 9, 569 (1937).
- A. W. W. A. COMMITTEE REPORT. Control of chlorination. *J. Amer. W. W. Assn.*, 35, 1315 (1943).

### 20. Sulfides (Total, Dissolved and Hydrogen Sulfide)

Three forms of sulfides are significant in sewage analysis:

(a) Total sulfides, which include all dissolved sulfides and also acid soluble metallic sulfides present as suspended matter.

(b) Dissolved sulfides.

(c) Un-ionized hydrogen sulfide.

The most accurate method for the determination of sulfides is by iodine titration, but this cannot be carried out directly in sewage because of interfering substances. It is necessary to evolve the sulfides in a stream of gas and collect them in zinc acetate solution before titration.

A more rapid method of sufficient accuracy for most purposes is the colorimetric method given under C.

#### A. SAMPLING

Samples must be taken with a minimum of aeration, for not only are sulfides volatilized by aeration, but any oxygen which is taken up destroys them by chemical action. Samples to be used only for total sulfide determination may be preserved by adding zinc acetate solution (B, 1.1) at the rate of 2 ml. per liter. This precipitates the sulfides as inert ZnS, and also prevents further sulfide generation. Determinations of dissolved sulfides, and analyses of samples not preserved with zinc acetate, must be commenced within three minutes of the time of sampling.

Samples to be used for determinations of total sulfides must contain a representative proportion of suspended solids.

#### B. TITRATION METHOD

##### 1. Reagents

1.1. Zinc acetate solution. Dissolve 240 g. of zinc acetate  $[\text{Zn}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 2\text{H}_2\text{O}]$  in 1 liter of distilled water.

1.2. Sodium thiosulfate solution. 0.025 N. (Part II, Sec. 10, C, 1.4-1.5, page 126.)

1.3. Iodine solution. 0.025 N. Dissolve 20-25 g. KI in a little water and add 3.175 g. iodine. After the iodine has gone into solution dilute to 1 liter and standardize against 0.025 N sodium thiosulfate.

1.4. Aluminum sulfate solution. Dissolve 24 g. of cp.  $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$  in distilled water to make 100 ml. of solution.

1.5. Sodium hydroxide solution. Dissolve 9 g. of cp. NaOH pellets in

distilled water to make 100 ml. of solution.

## 2. Procedure

2.1. Total sulfides. Introduce a measured volume of sample, usually approximately 500 ml., into a 1 liter aeration cylinder equipped with an alundum filter disc in the bottom, or a 1 liter wide-mouth bottle with a 2 hole stopper carrying a fritted glass diffuser tube and an outlet tube.

To the outlet, connect a 10 bulb absorption tube containing zinc acetate solution, or two 125 ml. conical flasks, each containing about 75 ml. of  $\text{Zn}(\text{C}_2\text{H}_3\text{O}_2)_2$  soln. with suitable connections to pass gas through them in series.

Acidify the sample with 10 ml. of concd.  $\text{H}_2\text{SO}_4$ . Pass  $\text{CO}_2$  or other inert gas (do not use air or  $\text{O}_2$ ) through the sample for one hour, or until suitable tests show that no more sulfide is coming over.

To the zinc acetate solution, add iodine solution well in excess of the amount necessary to react with the collected sulfides. (*One ml. of 0.025 N iodine is equivalent to 0.4 mg. of sulfide.*)

Add 5 ml. of concd.  $\text{HCl}$ , stopper and shake. (If two flasks are used, add nearly all of the iodine to the first flask, but half of the acid to each flask.)

Transfer the liquid to a beaker and back titrate with 0.025 N  $\text{Na}_2\text{S}_2\text{O}_3$ , using starch solution as indicator. For accurate results a blank should be run on the reagents, especially if the sulfide content is low.

Calculation:

ppm. total sulfide as S =  $[\text{ml. 0.025 N iodine} - \text{ml. 0.025 N Na}_2\text{S}_2\text{O}_3] \times 400 \div \text{ml. of sample.}$

2.2. Dissolved sulfides. Dissolved sulfides are determined on a sample from which the suspended solids have been removed by flocculating and settling.

Fill a 1 liter bottle with sample, flowing the liquid through the bottle after the manner of sampling for dissolved oxygen, in order to secure a sample which has had the least possible contact with air. Add 5 ml. of  $\text{Al}_2(\text{SO}_4)_3$  soln. and 5 ml. of  $\text{NaOH}$  soln. and stopper with no air bubbles under the stopper. Rotate back and forth about a transverse axis as vigorously as possible for at least a minute in order to flocculate the contents thoroughly. Allow to settle for 15 minutes, or until the supernatant liquid is reasonably clear. Siphon a suitable portion of this liquid into the evolution apparatus as described under 2.1. Acidify, and proceed as for total sulfides.

TABLE 16.—HYDROGEN SULFIDE FACTORS

pH	Factor	pH	Factor	pH	Factor
5.0	0.98	6.8	0.44	7.7	0.091
5.4	0.95	6.9	0.39	7.8	0.073
5.8	0.89	7.0	0.33	7.9	0.059
6.0	0.83	7.1	0.29	8.0	0.048
6.2	0.76	7.2	0.24	8.2	0.031
6.4	0.67	7.3	0.23	8.4	0.020
6.5	0.61	7.4	0.17	8.8	0.0079
6.6	0.56	7.5	0.14	9.2	0.0032
6.7	0.50	7.6	0.11	9.6	0.0013

2.3. Un-ionized  $\text{H}_2\text{S}$ . Determine the pH of the original sample. Determine dissolved sulfides by the foregoing procedure (2.2).

The concentration of un-ionized  $\text{H}_2\text{S}$  is found by multiplying the con-



centration of dissolved sulfides by the suitable factor obtained from Table 16. These factors are applicable at a temperature of 25° C. For temperatures below 20° C. or above 30° C. or for sewages having a mineral solids content exceeding 2000 ppm., suitable corrections should be made.

### C. COLORIMETRIC METHOD (METHYLENE BLUE)

This method is based on the fact that under suitable conditions para-amino-dimethyl-aniline, ferric ion, chloride ion, and sulfide ion react to produce methylene blue.

#### 1. Reagents

1.1. Stock amine sulfuric acid solution. Distill para-amino-dimethyl-aniline in an all-glass apparatus from which the air has been displaced by nitrogen or other inert gas. Mix 50 ml. of conc.  $\text{H}_2\text{SO}_4$  with 30 ml. of water and cool. Add to this 20 g. of the purified amine, stirring until solution is complete. Make up to 100 ml. with distilled water. This stock solution will discolor somewhat on standing, but its usefulness remains unimpaired. (Para-amino-dimethyl-aniline sulfate may be purchased, but its purity must be checked because its color-formation qualities vary.)

1.2. Amine-sulfuric acid test solution. Dilute 25 ml. of the stock amine-sulfuric acid solution with 975 ml. of 1:1 sulfuric acid.

1.3. Dilute sulfuric acid (1:1 solution).

1.4. Ferric chloride solution. Dissolve 100 g. of  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  in enough water to make 100 ml. of solution.

1.5. Ammonium phosphate solu-

tion. Dissolve 400 g. of di-ammonium phosphate,  $(\text{NH}_4)_2\text{HPO}_4$ , in enough water (805 ml.) to make 1 liter of solution.

1.6. Methylene blue solution. Prepare a solution of 1.00 g. of methylene blue per liter. This should be approximately the correct strength, but, because of variation of different samples of the dye, the solution should be standardized by carrying out analysis of samples of known sulfide concentration, or samples which are analyzed simultaneously by the titration method.

#### 2. Procedure

2.1. Total sulfides. Pipette 7.5 ml. portions of the sample into each of two test tubes which are accurately matched as to diameter. To the first tube add 0.5 ml. of the amine-sulfuric acid test solution. To the second tube add 0.5 ml. of dil.  $\text{H}_2\text{SO}_4$  (1:1).

To each tube add two drops of ferric chloride solution. Close tubes with the thumbs and mix by inverting slowly once or twice.

If sulfides are present, a blue color shows at once in the first tube; color development is complete in one minute. After 1 to 5 minutes add 1.6 ml. of the ammonium phosphate solution to each tube and mix.

Prepare droppers which deliver drops of the methylene blue solution weighing 48–52 mg. (In order to secure accurate results when measuring by drops, it is essential to hold the droppers steadily in a strictly vertical position, and to form the drops slowly enough so that the outside of the dropper is thoroughly drained before the drop falls.)

For greater accuracy the methylene blue solutions and the corresponding amount of water for the comparative tube may be measured with 1 ml. pipettes calibrated to 0.01 ml.

To the second of the two test tubes add drops or ml. of the methylene blue solution, and add the same amount of water to the first tube in order to keep the volumes equal. Determine the amount of methylene blue solution necessary to bring the two tubes to the same color. If the methylene blue solution is of proper strength, one drop will correspond to 1 ppm. of sulfide as "S" or 0.1 ml. will correspond to 2 ppm. of sulfide. To determine fractional parts, dilute some of the methylene blue solution to one-tenth strength.

2.2. Dissolved sulfides and hydrogen sulfide. Determinations of dissolved sulfides by the colorimetric method are made on samples from which the suspended solids have been removed by the procedure described under B, 2.2, except that a bottle of 100 ml. or 250 ml. may be used for flocculating, instead of a liter bottle. Reduce the amounts of flocculating chemicals in proportion to the volume of the bottle.

Hydrogen sulfide is calculated as described in B, 2.3.

### 3. *Limitations of the Colorimetric Method*

The amine-sulfuric acid test solution contains 5 g. of amine per liter. When prepared in this way, it may be used to test sulfide concentrations up to 20 ppm. Color development is deficient at higher concentrations, and if the sulfide content is very high

(several hundred ppm.) color formation may be completely inhibited.

If desired, higher concentrations of amine may be used to test concentrations above 20 ppm., but because of the color intensity this is not very satisfactory. An alternate method is to dilute immediately before testing.

For example, measure 6 ml. of water into the test tube, add 0.5 ml. of solution 1, pipette in 1.5 ml. of sample, and finish as in the normal procedure, multiplying the final result by 5.

A slight interfering color may be noted at sulfide concentrations approximating 0.1 ppm. This may be eliminated if necessary by reducing the amount of amine in the reagent to 3 g. per liter, and extending the time for color development to 5 minutes. The reagent so prepared should not be used for testing sulfide concentrations above 5 ppm.

Concentrations of sulfite and thio-sulfate in excess of 10 ppm. cause deficient color development, but by increasing the amount of ferric chloride from 2 drops to 6 drops and extending the reaction time to 5 minutes the method may be used in the presence of 50 ppm. of these compounds.

### BIBLIOGRAPHY

- RUDOLFS, W., AND BAUMGARTNER, W. H. Studies of hydrogen sulfide formation in sewage. *Ind. Eng. Chem.* 24, 1152 (1932).  
POMEROY, R. D. The determination of sulfides in sewage. *Sew. Works J.*, 8, 572 (1936).  
POMEROY, R. D. Hydrogen sulfide in sewage. *Sew. Works J.*, 13, 498 (1941).

### 21. Grease

Grease is defined as that material which is extracted from an acidified

sample of sewage by petroleum ether (B.P. 40°–60° C.).

### 1. Reagents

- 1.1. Hydrochloric acid, concd.
- 1.2. Petroleum ether, B.P. 40°–60° C.
- 1.3. Cotton discs, washed with solvent and dried.
- 1.4. Filter paper—No. 1 Whatman 9 cm. or equal.

### 2. Procedure

Strongly acidify (pH. 1.0) a liter sample with concd. HCl. Bring to boil and boil for two minutes. Cool and chill in refrigerator (50° F. or lower) for at least two hours.

Prepare a 9 cm. filter paper overlaid with grease-free cotton disc (cut from absorbent cotton to fit filter) extracted with petroleum ether and dried.

Filter the chilled sample through the cotton disc. Wipe the sides and bottom of the beaker carefully with a pad of grease-free cotton taking care to collect all solid material; add to filter.

Dry the filter paper and cotton pad in an evaporating dish in a hot air oven at 103°, for 30 to 40 minutes.

Roll filter paper and cotton pad to fit in a suitable extraction thimble and insert in thimble.

Transfer filtrate to a separatory funnel. Shake with 50 ml. or more of solvent. Run off the water layer and filter the ether layer through the Soxhlet thimble into a weighed extraction flask.

Extract in Soxhlet apparatus at rate of 8 to 10 cycles per hour for 3 to 5 hours.

Drive off ether from extraction flask on water bath and dry in oven at 103° C. for 15 minutes.

Draw off last traces of ether vapor by inserting a tube connected with vacuum pump into flask while still warm.

Cool in desiccator for one hour and weigh.

ppm. total grease = gain in weight of flask in gms.  $\times$  1000.

### BIBLIOGRAPHY

- OKUN, D., HURWITZ, E., AND MOHLMAN, F. W. Investigation of methods for the determination of grease in sewage and sludge. *Sew. Works J.*, 13, 485 (1941).  
 HATFIELD, W. D., AND SYMONS, GEORGE E. The determination of grease in sewage. *Sew. Works J.*, 17, 16 (1945).

### 22. Collection of Sludge and Mud Samples

Great care must be taken in sampling sewage sludges, sludge banks, muds, etc. No definite procedure can be given, but every possible precaution should be taken to obtain a representative sample whether from a tank, a sludge pile or from the bottom of a river.

When analysis cannot be made immediately, the sludge or mud samples should be stored on ice. The samples cannot be preserved with chloroform if grease is to be determined. Mud samples may be preserved with 5 g. of sodium benzoate, or one ml. of concd.  $\text{H}_2\text{SO}_4$  to each 80 g. of mud.

In the following sections wherever the word sludge or sewage sludge is used the statement or direction is to apply to muds as well unless otherwise stated.



## BIBLIOGRAPHY

JEWELL, M. E. Experiments on the preservation of mud samples. *Ill. State Water Survey, Bull. 16*, 206 (1920).

## 23. Physical Tests on Sludge

Color, odor, and physical appearance shall be described as best suits the individual case, no standard method being recommended. Temperature shall be recorded in degrees Centigrade.

## 24. Specific Gravity of Sludge

## 1. Procedure

Weigh (to the nearest 0.1 g.) an empty wide-mouthed flask or bottle of about 250 ml. capacity. Weigh the same flask filled completely with distilled water, and again filled completely with the sample.

Calculation:

Spec. grav. = [weight of sludge or mud] ÷ weight of same volume of distilled water.

Record the specific gravity to the third place.

In case the mud does not flow readily, add as much of it to the bottle as possible, without exerting pressure, and weigh. Fill bottle with water and weigh again, making sure that all entrained air bubbles have escaped.

Calculation:

Spec. grav. of mud = [weight of mud] ÷ (weight of water to fill a bottle - weight of water added to mud).

## 25. Suspended Solids of Sludges and Aeration Tank Liquor—Aluminum Dish Method

## 1. Apparatus

1.1. Aluminum dish, 92 mm. inside diameter, 25 mm. high with bottom perforated similar to a Büchner funnel.

1.2. E D filter paper no. 615 or equal, 90 mm. in diameter.

1.3. Sponge rubber ring: 93 mm. outside diameter, 75 mm. inside diameter, thickness about 3 mm.

1.4. Büchner funnel: no. 2A, inside diameter at bottom, 93 mm.

1.5. Standard filter flask with side tube 1000 ml. size.

## 2. Procedure

Dry dish and filter paper in oven. Cool in desiccator and weigh. Wet the filter paper, place the dish with paper on rubber ring in Büchner funnel and apply about 20 inches of vacuum to the flask.

Immediately add 50 or 100 g. of sludge to the dish and after the water has been extracted, dry in oven for about 30 minutes at 103° to 105° C. Cool in desiccator and weigh.

The test is applicable to aeration tank mixed liquor in which case it is reported as per cent suspended solids, or to heavier sludges such as return sludges when it is reported as per cent of dry sludge solids.

## BIBLIOGRAPHY

SMITH, J. I. Investigation of rapid methods for sludge solids estimation. *Sew. Works J.*, 6, 908 (1934).

## 26. Settleability of Activated Sludge

## 1. Procedure

Allow 1000 ml. of activated sludge to settle in a 1000 ml. graduated cylinder. Record the volume occupied by the sludge at various intervals of time (10, 20, 30, 45, 60, etc. minutes), plot these volumes against the time and obtain a curve indicating the rate of settling. For plant control a 30

minute settling period is rather generally used.

## 27. Sludge Volume Index (S. V. I.)

### 1. Procedure

The sludge volume index is the volume in ml. occupied by 1 g. of activated sludge after settling the aerated liquor for 30 minutes. A 1 liter sample is collected at the outlet of the aeration tanks, settled 30 minutes in a 1000 ml. graduated cylinder and the volume occupied by the sludge reported in per cent or ml. The sample is thoroughly mixed, or an original sample taken, and the suspended solids determined and reported in per cent by weight or parts per million.

Calculation:

$$\text{S. V. I.} = \frac{\text{per cent settling by volume}}{\text{per cent suspended solids}} \times \text{ml. settling sludge} \times 1000 \div \text{ppm. suspended solids.}$$

## BIBLIOGRAPHY

- THERIAULT, E. J., AND WAGENHALS, H. H. Studies of representative sewage plants. *U. S. Pub. Health Bull.*, 132, 24 (1923).  
 MOHLMAN, F. W. Editorial. *Sew. Works J.*, 6, 119 (1934).

## 28. Sludge Density Index (S. D. I.)

The sludge density index is the reciprocal of the "sludge volume index" multiplied by 100, and is calculated from the same data as follows:

$$\text{S. D. I.} = \frac{\text{per cent suspended solids} \times 100}{\text{per cent settling by volume}} = \text{ppm. suspended solids} \div \text{ml. settled sludge} \times 10 = 100 \div \text{S. V. I.}$$

## BIBLIOGRAPHY

- THERIAULT, E. J., AND WAGENHALS, H. H. Studies of representative sewage plants. *U. S. Pub. Health Bull.*, 132, 24 (1923).  
 DONALDSON, W. Some notes on the operation of sewage treatment works. *Sew. Works J.*, 4, 48 (1932).

## 29. Reaction (Acidity, Alkalinity and pH) of Sludges

Determine the acidity, alkalinity and pH value of the liquor that separates from the sludge on standing. Both acidity and alkalinity titrations are made using a tall cylinder and plunger as described for titrating carbon dioxide (Part II, Sec. 14, 2, page 146).

### A. ACIDITY (TO PHENOLPHTHALEIN)

#### 1. Procedure

Measure into a 90-100 ml. cylinder (hydrometer jar) 50 ml. of supernatant liquor from the sample. Care must be taken to minimize the loss of  $\text{CO}_2$  in these operations. Add 3 drops of phenolphthalein indicator solution and titrate with 0.05 N NaOH soln. to the first permanent pink color. A plunger type of stirring rod is used.

Calculation:

$$\text{ppm. acidity to phenolphthalein expressed as CaCO}_3 = \text{ml. 0.05 NaOH} \times 50.$$

$$\text{acidity in milliequivalents} = \text{ml. 0.05 N NaOH.}$$

### B. ALKALINITY

#### 1. Procedure

Measure 50 ml. of the supernatant liquor (see acidity above) in a glass cylinder. Add 3 drops of phenolphthalein and titrate with 0.05 N  $\text{H}_2\text{SO}_4$  until pink color has disappeared. Record ml. 0.05 N acid used as P.

Add 3 drops of methyl orange and continue titration until the solution is a faint pink. Record the total ml. of 0.05 N acid used, including that for the phenolphthalein titration, as T.

Calculation: When 50 ml. of supernatant sludge liquor is used

ppm. alkalinity to phenolphthalein as  $\text{CaCO}_3 = P \times 50$ .

ppm. alkalinity to methyl orange as  $\text{CaCO}_3 = T \times 50$ .

To express these results in milliequivalents of alkalinity, use the factor 1 instead of 50 in the above calculations.

### C. pH VALUE

The pH of the liquor in normal sewage sludge, particularly digested sludge, is dependent to a considerable extent on the carbon dioxide equilibrium. Any technic which allows a loss of carbon dioxide from the liquid will give pH results that are too high.

#### 1. Procedure with Glass Electrode

The standard method for determining the pH value shall be the glass electrode procedure, Part I, Sec. 11, page 28. If extremely accurate results are not desired colorimetric methods may be used (Appendix I, Sec. 3, page 211).

### BIBLIOGRAPHY

- FAIR, G. M., AND MOORE, E. W. Determining the pH of sewage sludges. *Sew. Works J.*, 1, 3 (1930).  
 HATFIELD, W. D., AND MORKERT, K. Determining the pH of sewage sludges. *Sew. Works J.*, 6, 246 (1934).

### 30. Moisture and Solids in Sludge

#### A. MOISTURE AND TOTAL SOLIDS

##### 1. Procedure

Weigh 25 to 50 g. of sludge to the nearest 0.1 g. in a tared evaporating dish. Evaporate to dryness on a water bath, dry at 103° C. for 1 hour, cool in desiccator and reweigh. If the

material contains 15 or more per cent solids, it may be necessary to dry for a longer period of time. This is particularly true if the material is high in organic or grease content. With samples of this type, evaporating in an oven at 103° C. overnight or drying to constant weight may be necessary but by this prolonged heating in either technic, there will be loss of volatile organic matter and ammonium carbonates.

### B. VOLATILE SOLIDS

(Includes volatile inorganic salts)

The determination of both total and volatile solids is subject to error due to the loss of ammonium carbonate and volatile organic matter while drying (Part II, Sec. 29, A, 1, page 158), making the total solids lower than they should be.

#### 1. Procedure

Ignite the residue from the determination of moisture in an electric muffle at 600° C. for 60 minutes, avoiding loss of solids by decrepitation. Cool in a desiccator and reweigh. Report results as per cent ash and volatile solids.

### 31. Nitrogen in Sludge

#### A. TOTAL NITROGEN

##### 1. Procedure

Dry a sample of sludge or mud in a drying oven, at 103° C., grind thoroughly to a fine powder and dry again for 30 minutes at 103° C.

Weigh accurately 1 g. of dried sludge or 1 to 5 g. of dried mud into a 500 ml. Kjeldahl flask, add 20 ml. of  $\text{H}_2\text{SO}_4$ , 1 ml. of 10 per cent  $\text{CuSO}_4$



soln. and 5–10 g. of  $K_2SO_4$  or  $Na_2SO_4$ , mix thoroughly, digest slowly until frothing ceases and then for 30 minutes after the liquor becomes clear.

Cool, dilute to 300 ml. with ammonia-free water, and proceed as directed in Part II, Sec. 5, page 118, except that 0.5 N instead of 0.05 N  $H_2SO_4$  is used, and 0.5 N NaOH soln. is used for back titration. One ml. of 0.5 N acid is equivalent to 0.007 g. of N. The results shall be expressed as per cent N. (The boric acid method may also be used if desired (Part II, Sec. 5, 2, page 118).

The above method as it stands is far from being the total nitrogen of the *wet sludge*. At best it is Kjeldahl nitrogen minus ammonium bicarbonate or carbonate lost on drying. A more rational method for determining the total nitrogen in wet sludge is to determine the ammonia nitrogen and organic nitrogen as in B and C of this section.

## B. AMMONIA NITROGEN

### 1. Procedure

Rapidly weigh approximately 20 g. of wet sludge (5 per cent solids) to 0.1 mg. in a weighing bottle or crucible. Wash sample into a 500 ml. Kjeldahl flask with distilled water or ammonia-free water, dilute to 250 ml. and proceed as directed in Part II, Sec. 4, C, 3, page 117, distillation procedure, except that 100 ml. of distillate is collected in standard  $H_2SO_4$  and the excess acid titrated to the methyl red end point. (Distillation into boric acid may be used.) A piece of paraffin added to the flask will prevent frothing. Calculate in parts per million N.

## C. ORGANIC NITROGEN

### 1. Procedure

To the residue from the ammonia determination add 1 ml. of 10 per cent  $CuSO_4$  soln., 5–10 g. of  $Na_2SO_4$  or  $K_2SO_4$  and 20 ml. of concd.  $H_2SO_4$ , digest and proceed as directed in Part II, Sec. 5, 2, page 118. Calculate as per cent N of the total solids, the latter determined on a separate sample.

### 32. Grease in Liquid Sludge

Grease is defined as that material which is extracted from an acidified sample by petroleum ether (B.P.  $40^\circ$ – $60^\circ$  C.)

#### 1. Reagents

1.1. Hydrochloric acid, petroleum ether, cotton discs, and filter paper as given in Part II, Sec. 21, 1.1–1.4, page 156.

#### 2. Procedure

Weigh a sample large enough to yield approximately 50 mg. of grease. Strongly acidify the sample (pH 1.0) with concd. HCl. Bring to a boil and boil for two minutes. Cool and chill in refrigerator ( $10^\circ$  C. or lower) for at least two hours.

Prepare a 9 cm. filter paper overlaid with grease-free cotton discs (cut from absorbent cotton to fit filter), extracted with petroleum ether and dried.

Filter the chilled sample through the cotton disc. Wipe the sides and bottom of the beaker carefully with a pad of grease-free cotton, taking care to collect all solid material; add to filter.

Dry the filter paper and cotton pad in an evaporating dish in a hot air oven at 103° C. for 30 to 40 minutes. Roll filter paper and cotton pad to fit in a suitable extraction thimble and insert in thimble.

Extract in Soxhlet apparatus at rate of 8 to 10 cycles per hour for 3 to 5 hours.

Drive off the petroleum ether from extraction flask on water bath and dry in oven at 103° C. for 15 minutes. Draw off last traces of petroleum ether vapor by inserting tube connected to vacuum pump into flask

while still warm. Cool in desiccator for one hour and weigh.

Determine the per cent of dry solids on a separate sample.

Calculation:

per cent total grease =  $\frac{\text{grain in weight of flask in gm.} \times 100}{\text{dry weight of sample in gm.}}$

#### BIBLIOGRAPHY

- OKUN, D., HURWITZ, E., AND MOHLMAN, F. W. Investigation of methods for the determination of grease in sewage and sludge. *Sew. Works J.*, 13, 485 (1941).  
HATFIELD, W. D., AND SYMONS, GEORGE E. The determination of grease in sewage. *Sew. Works J.*, 17, 16 (1945).

## PART III

### MICROSCOPICAL EXAMINATION OF WATER, SEWAGE SLUDGE, AND BOTTOM SEDIMENTS

#### 1. Definition and Scope of Water Examinations

The microscopical examination of water embraces a qualitative analysis of the kinds of microscopic organisms present, a quantitative estimate of their number, or bulk, and a brief survey of the crystalline and amorphous matter. Such information is important in gauging water quality and may serve any one or more of the following purposes:

a. To explain the cause of color and turbidity and the presence of objectionable odors and tastes in water and to indicate methods for their removal.

b. To aid in the interpretation of the chemical analysis.

c. To identify the source of a water that is mixing with another.

d. To explain the clogging of pipes and filters and to aid in the design and operation of water works.

e. To indicate pollution by sewage or industrial wastes.

f. To indicate the progress of the self-purification of streams and other bodies of water.

g. To aid in explaining the mechanism of biological sewage treatment methods.

h. To aid in the study of the food of fish, shellfish, and other aquatic organisms.

i. To determine whether or not ground water is contaminated by unfiltered surface water.

The term "microscopic organisms" shall include all organisms that are microscopic or barely visible to the naked eye, with the exception of the bacteria. The term "Plankton" is synonymous with it when used in a broad sense to designate not only free-floating forms, but also, those minute forms that are ordinarily attached to shore or bottom. Included with the microscopic organisms are such groups among the plants as the Diatomaceae, the Chlorophyceae, the Cyanophyceae, and the fungi; and among the animals the protozoa, the rotifera, the crustacea, the porifera, the bryozoa, and numerous small worms and insect larvae.

Clumps of organic matter, silt, cast molts of water organisms and headless stalks of such organisms as *Carchesium*, shall be considered as amorphous matter. The prevailing kinds and the relative qualities of this amorphous matter should be recorded in many cases for their value in characterizing a water.

#### 2. Apparatus for Water Examination

##### A. DEVICES FOR COLLECTING SAMPLES

Use a two-liter\* glass bottle or special sampler which will collect water at any desired depth. Such

\* Some investigators prefer to use smaller samples (around 250 ml.); however, this procedure is not to be recommended inasmuch as the larger samples make for greater accuracy.



samplers are illustrated and described in references in the bibliography.

## B. DEVICES FOR CONCENTRATION OF SAMPLE

### 1. *Sedgwick-Rafter Method*

1.1. Filtering funnels. A cylindrical funnel is required; the diameter should be approximately two inches at the top, with a straight side for about nine inches, narrowing over a distance of about three inches to a bore of one-half inch diameter and terminating in a straight portion of this diameter about  $2\frac{1}{2}$  in. in length. The capacity of this funnel is 500 ml. It shall be fitted at the bottom with a tightly fitting, one-hole rubber stopper, and the stopper shall contain a small glass U-tube, the outer arm of which extends one inch above the small end of the stopper. This tube prevents draining of the sand after filtration.

1.2. Cloth discs. Cloth discs, about three-eighths inch in diameter, are needed to support the sand in the funnel. These are cut preferably from silk bolting cloth having about 200 meshes to the inch, although similar linen cloth may be used.

1.3. Filtering sand. White sand is required as the filtering medium. This may be Berkshire or Ottawa sand, ground quartz or white beach sand. It should be washed and screened, only that portion being used which passes a U. S. Series No. 60 screen and is retained on a No. 120 screen.

### 2. *Centrifuge Method*

2.1. Continuous centrifuge. A continuous electric centrifuge similar

to the Foerst type is required. (See Figure 12.) The centrifuge should have a speed of approximately 20,000 rpm. and should be light enough to be portable.

### 2.2. Automatic feeding funnel.

An ordinary glass funnel with a maximum diameter of six inches is needed. This funnel should be drawn out into a tip which allows a liter of water to pass in six or seven minutes, or a calibrated tip may be attached. The integral tip is much to be desired inasmuch as it renders adequate cleaning of the apparatus between samples more certain.

2.3. Support for sample bottle and feeding funnel. For portable equipment a heavy ring stand and three rings are needed. One ring with a diameter of 7.5–10 cm. serves as a support for the feeding funnel. Another ring, the size of which depends upon the type of sample bottle being used, is incomplete for one-fourth of its circumference. This ring serves as a support for the sample bottle which rests upon it in an inverted position with its neck and mouth below the upper edge of the funnel. The removal of a sector of this supporting ring facilitates the insertion of a full sample bottle.

The third ring is slipped over the base of the inverted bottle to keep it in position, precluding any possibility of tipping.

In the permanent laboratory a heavy iron rod with two right angle bends four inches apart is used in place of the ring stand. One end of this rod is pointed down and fastened to the wall, and the projecting, upwardly directed portion is used for

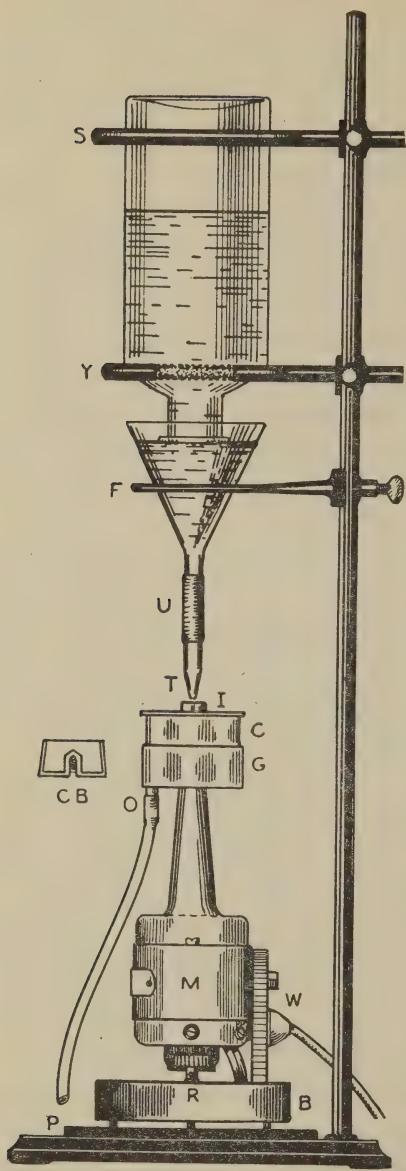


FIG. 12. FUNNEL AND CENTRIFUGE ASSEMBLY FOR CONCENTRATING ORGANISMS. *P*, pad, rubber or felt; *B*, base; *M*, motor; *R*, rheostat; *W*, motor terminals; *G*, guard; *C*, cover; *CB*, cross section of bowl; *I*, inlet tube; *O*, outlet tube; *T*, tip regulating flow; *U*, rubber tubing; *F*, funnel support; *Y*, incomplete ring for bottle support; *S*, guard ring.

the attachment of the rings mentioned above.

Manual feeding may be employed if the level of the water in the feeding funnel is kept constant.

### 3. Alternative Methods

Other forms of apparatus for filtration are allowable if their accuracy, under the conditions used, is as great as that of the apparatus already described. The method of Kofoid employing an ordinary glass funnel lined with a hard surface filter paper (No. 575 Schleicher and Schull or No. 50 Whatman), the sling filter method, or the sedimentation method involving the use of a siphon may be substituted under special conditions.

The use of plankton net collection of not less than 50 liters, and preferably 100 liters, is recommended as a supplement to smaller collections when a thorough survey is to be made. By means of such large samples, trustworthy information on the abundance and kinds of larger forms of microscopic life may be obtained.

Ordinarily, even in a 2 liter sample these forms are too scarce to assure accurate results when quantitative determinations are desired. The concentration may be accomplished by filtering the required volume of water through bolting cloth as the straining medium. This bolting cloth, when thoroughly shrunk, has 6200 meshes to the square centimeter, or 40,000 meshes per square inch.

## C. APPARATUS FOR EXAMINATION OF WATER SAMPLES

### 1. Microscopes

1.1. Compound microscope. A binocular-type compound microscope

is preferable although the monocular type may be used when only a few samples are examined. The microscope should be equipped with a detachable mechanical stage capable of moving all parts of a standard counting cell past the aperture of the objective. It should also have an adequate combination of oculars and objectives consisting of  $7.5\times$  and  $10\times$  oculars and the following objectives:

Oil immersion objective  
 4 mm. objective  
 8 mm. " "  
 16 mm. " "

1.2. Low power binocular microscope. In counting larger organisms such as Cyclops, and in the study of organisms which occur in bottom sediments, this microscope is indispensable. It should be equipped with  $10\times$  and  $15\times$  oculars, and with  $1.5\times$  and  $7.5\times$  objectives. So

equipped, it bridges the gap between the hand lens and the compound microscope.

## 2. Counting Cell

A counting cell shall be used consisting of a brass or glass rim closely cemented to a plate of optical glass, or plate glass. The depth of this cell shall be 1 mm. The shape and size of the cell, excepting its depth, are not prescribed; but a rectangular cell with inside dimensions  $20\times 50$  mm. and 1 ml. capacity is convenient. A cover glass, preferably No. 3, of such size as to cover the cell and its rim completely is required.

## 3. Ocular micrometer

3.1. For field limitations. The microscope must be fitted with an ocular micrometer (Fig. 13) ruled in order to define the limits of the microscopic field. This micrometer is so

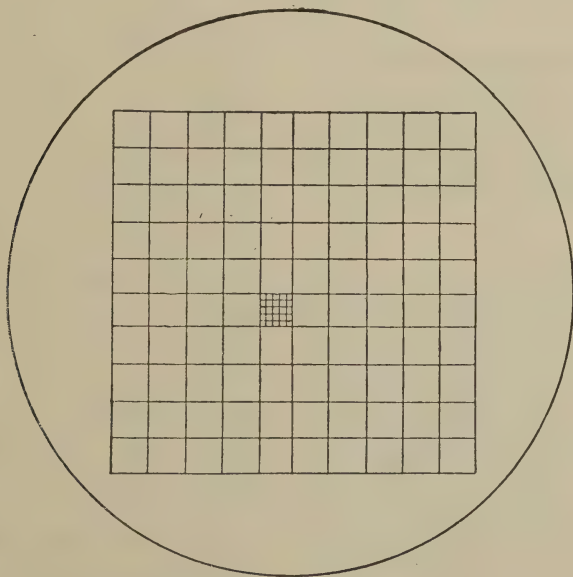


FIG. 13. OCULAR MICROMETER RULING.



dimensioned that the area on the stage covered by it is exactly one square mm. if a certain combination of objective, eyepiece and tube length is used. This combination must be determined by trial with a stage micrometer.

Tube length is not usually an adjustable feature in the binocular type of compound microscope, hence the exact coverage of the ocular field must be measured, and if it exceeds or falls below one square mm., a factor must be developed to convert the results obtained to that represented by the standard area.

When the outside limits of the ruling on the micrometer cover a square mm. on the stage, the smallest square on the micrometer will cover an area of  $20 \times 20$  microns or 400 square microns. This is known as the "Areal Standard Unit." The "Volumetric Standard Unit" has the same area and a thickness or depth of 20 microns. Its volume is thus 8000 cubic microns.

3.2. For measurement of objects. A ruled scale is needed which may be placed in the ocular of the microscope where its image is superimposed on that of the organism examined.

#### 4. Stage Micrometer

This is a standardized accurately ruled micrometer scale mounted on a glass slide. The lines are engraved on glass and the finest marks should be not less than 0.01 millimeter apart. It is needed for the calibration of the field limiting ocular and standard ocular as well as to establish the field areas of the various objective and ocular combinations.

### 3. Collection of Water Samples

Sampling points shall be carefully selected with a view to obtaining as representative a dispersion of organisms as possible and to excluding floating debris, mud, and extraneous material. Sampling points should be essentially the same as those of chemical and bacteriological samples, when the latter are included in the study, in order to afford reasonable correlation of results.

Samplers used to collect chemical, biochemical and other samples may be employed and collections should coincide as far as possible with those made for the purposes mentioned. If no sampler is available and a surface sample will be adequate, a glass bottle may be used. For this purpose, a clean bottle (not necessarily sterile) holding preferably two liters (not less than one liter) shall be thrust down about one foot beneath the surface of the water, mouth downward, with stopper removed.

After it is inverted, it shall be allowed to fill. Unless the sample is to be examined in a fresh condition it should be preserved immediately by the addition of 40 ml. of formalin \* to each liter collected. Samples of scum growths and attached forms may be taken by special techniques, but should be kept separate from the quantitative material.

When samples from various depths are required, special sampling devices must be used. Samplers which will collect under a variety of conditions are illustrated and described in references in the bibliography.

\* Commercial formalin (37-42 per cent formaldehyde).

#### 4. Concentration of Water Samples

##### 1. Procedure

If the number of organisms in one ml. of water is fairly large (25 or more), it is possible to subject the sample to "direct" examination. If not, concentration of the sample becomes necessary.

1.1. Concentration by the Sedgwick-Rafter method. Filtration shall be carried out as soon as possible after collection of the sample. If the sample is kept cool, three or four hours may safely intervene, but for longer periods and at high (summer) temperatures it is desirable to preserve the sample as directed in Sec. 3, above.

Prepare the Sedgwick-Rafter filter for use, first inserting the glass U-tube not too snugly in the large end of the rubber stopper, then cover the moistened small end with a disc of bolting cloth and place the whole firmly in the lower end of the funnel. The latter should be perfectly clean on the inside.

Pour sand into the funnel to form a layer one-half inch deep on top of the disc. Add 5 to 10 ml. of distilled water to wash down any sand on the walls of the funnel and to drive the air from the sand. As the distilled water filters through the sand, tilt the funnel from side to side to permit the escape of air.

Mix the sample well, but do not shake it violently, otherwise fragile organisms will be disintegrated. Measure out 250 to 1000 ml., according to the density of microscopic organisms in the sample, in a graduate or flask, and pour slowly into the fun-

nel, holding the latter in a slanting position so as not to disturb the sand.

Allow the water to filter through the sand, using moderate suction, if need be, to hasten filtration. Wash down the side occasionally with distilled water from a wash-bottle. After the water has reached the level of the U-tube, disconnect the suction, if employed, and carefully remove the U-tube to allow most of the remaining water to drain through the sand.

As soon as the sand has drained, transfer the funnel to a horizontal position, remove the stopper slowly with a twisting movement, then raise the funnel to a vertical position inside a small beaker. The plug of sand usually falls into the beaker. Wash down the walls of the funnel with 5 to 20 ml. of distilled water (the amount varying with the concentration to be obtained) measured with a pipette, collecting it in the beaker that contains the sand and organisms. The container is then gently shaken to detach organisms from the sand grains.

Allow a moment for the coarse sand to settle, then decant promptly into a beaker. A second washing, with an additional 5 ml. of water, is usually necessary. If mixing has been thorough, any water remaining in the sand will have the same concentration of organisms as the water in the beaker.

If the concentration is to be preserved for future examination, the funnel originally may be washed down with a 3 to 10 per cent solution of formalin, or the formalin may be added to the concentrate and the latter made up to a definite volume; some multiple of 5 is convenient.

1.2. Concentration by the centri-

fuge method. Because of the rapidity with which concentration may be accomplished by this method, not less than 2 liters of sample should be used. If the water contains highly buoyant organisms which float near the surface, where they frequently form a scum, the sample should be centrifuged twice. *Aphanizomenon* has been known to cause trouble in this way and it is possible that other somewhat similar forms may sometimes be involved. In the case of *Aphanizomenon*, the first centrifuging removes about one-half of the filaments, while the second operation collects substantially all of the remainder. With regard to other organisms usually considered in a plankton catch, approximately 98 per cent are removed at the first centrifuging.

Where time permits, another method of concentrating highly buoyant organisms is available, namely, allowing the sample to stand until these light forms produce a scum which is skimmed off and added to the centrifuge concentrate.

Assemble self-feeding arrangement for use (Fig. 12). First insert the calibrated tip of the funnel slightly below the upper rim of the central collar or inlet tube of the centrifuge, centering the tip of the funnel over the rotor cup of the centrifuge so that even distribution is assured, then move the incomplete ring down to within an inch or an inch-and-a-half of the top of the funnel. The centrifuge is then set in motion and the sample bottle from which the stopper has been removed is quickly inverted and slipped to position above the funnel in such a manner that the neck

of the bottle is below the upper edge of the funnel. The sample is thus fed into the funnel at a rate equivalent to that of the discharge of the calibrated tip, and after the third or supporting ring has been slipped into place, the apparatus may be left to itself until the entire sample has been centrifuged.

After the sample is concentrated, the bowl is removed from the centrifuge and the organisms are gently rubbed off its sloping sides by means of a soft rubber policeman, or a match, the tip of which has been charred by burning and rubbed smooth on a hard towel. Ordinarily the concentrate has a volume of 4 or 5 ml.

If the concentrate is to be preserved for future examination, this quantity is diluted to a proportional amount of the original sample by the addition of a 3 to 10 per cent solution of formalin; some multiple of 5 is convenient. Made from commercial formalin, which contains 37 to 42 per cent formaldehyde, a 3 per cent formalin solution thus contains 1.11 to 1.26 per cent formaldehyde.

The sample is transferred to a small, properly labeled sample bottle and is then ready for examination. If the sample is very turbid, it may be diluted further at any time to permit ready microscopic examination.

When the concentrate has been removed, all parts of the centrifuge and self-feeding arrangement, which actually come in contact with the water, are thoroughly cleaned before another sample is put through the machine.

1.3. Concentration by alternative methods. Silk net filtration method.



Fifty to 100 liters of sample are strained through fine silk bolting cloth which has 40,000 meshes per square inch.

The water should be carefully measured by dipping or pumping into a calibrated pail or other container. The net should be completely immersed in water, except for its upper margin, when the water to be strained is poured in. This will prevent forcing certain organisms through the mesh by hydrostatic pressure and insures gentle, efficient straining.

If the net tends to clog, grasp it lightly immediately below the top ring, gather fabric into one hand, and gently shake the whole while keeping it submerged. This tends to loosen the particles which are stopping the mesh openings and drops them into the plankton bucket at the lower end of the net.

When the full volume has been strained, wash down the inside of the net thoroughly, rinsing the organisms and debris into the bucket. Disconnect the latter and wash its contents into the sample bottle by withdrawing the central plug valve and spraying with clean water from a soft rubber ear syringe. Add formaldehyde solution to make a concentration of three per cent and place an identifying label inside the specimen bottle.

The silk net used in this method is fragile and perishable. It must have constant care and attention to be efficient. After each use, wash thoroughly by turning inside out and running water through in the reverse direction. Air dry with as little delay as possible to prevent rotting and pack away in such a manner that

metal parts cannot wear a hole or that folds do not always occur in the same place and produce a crack. Before straining a new sample, wash once more in order that contamination may not be carried over to cause difficulty in the interpretation of the second sample.

## 5. Examination of Water Samples

### A. CALIBRATION OF THE MICROSCOPE

Microscope eyepieces are often fitted with discs bearing parallel, engraved, equidistant lines or engraved, subdivided squares which are designed to measure microscopic objects or accurately delimit microscope fields. Before these ocular micrometers can be used, however, they must be carefully calibrated in combination with each objective.

The ocular micrometers are calibrated by measuring an object of known dimensions, thus determining the value of each subdivision by reference. A glass slide, on which an accurately ruled scale has been engraved, is placed on the microscope stage and serves as the object of known dimension.

The procedure consists simply in determining how many intervals on the ocular micrometer are required to cover one or several intervals on the scale of the stage micrometer. With the ocular and stage micrometers parallel and in part superimposed, a line at one end of the eyepiece scale is selected and matched with a similar line of the stage micrometer scale. The two are then carefully examined and it is observed

that the lines also coincide at another point.

If the distance between lines on the stage micrometer is known, the linear value of each ocular division can be determined by reference. For example, if the smallest interval on the stage micrometer is 0.01 mm. and ten of these (0.1 mm.) intercept 25 divisions on the ocular scale, 1 ocular division =  $0.1 \text{ mm.} \div 25$  or 0.004 mm. (4 microns).

When high-power objectives are calibrated, the stage micrometer lines are magnified to a point where they have appreciable width. As a result, the calibration procedure must be modified by placing an ocular line alongside of, rather than end-to-end with the stage micrometer lines.

Single observations will not suffice to establish a true calibration. The average of a large number of observations must be used. The data obtained will be more readily available and usable if a graph is prepared in which ocular scale dimensions are plotted against the linear distance they represent on the stage (see Fig. 14). By reference to this chart, the length in microns of any given object examined may be determined quickly.

## B. SELECTION OF ALIQUOT PORTION

The portion taken from the concentrated sample should be representative and the examination should include a sufficient number of organisms to insure accuracy.

Shake the concentrated sample gently but in such a manner that complete mixing will occur and that clumps may be broken up. It is better that long filamentous forms be

broken into shorter lengths than to have a poor distribution in the counting chamber.

Place the cover glass obliquely across the cell. By means of a pipette, withdraw one ml. of material from the sample bottle before the motion of the sample induced by mixing has ceased. Introduce the concentrate half at each open corner of the cell. When carefully done, this will cause the cover slip to rotate automatically into a position of complete coverage of the cell. Allow the latter to stand for four to five minutes, long enough to permit organisms to settle to the bottom or rise to the top.

## C. EXAMINATION AND ENUMERATION OF ORGANISMS

### 1. Procedure

Examine under the microscope in three ways. First, the most abundant forms are counted by examining a number of standard fields, next, a strip extending the whole length of the cell is examined for organisms which are less numerous and, finally, the whole cell is examined for large forms and those very limited in number. In all cases, examine the full depth of the cell to include floating forms.

1.1. Field count. The "field count is made with a 16 mm. objective and a  $7.5 \times$  ocular which has a ruled ocular of the type described in Part III, 2, C, 3.1, page 165. Where the distribution of organisms in the sample is relatively uniform, the number of fields examined will depend upon the concentration of organisms which prevails. "... The same accuracy is obtained by counting the same number of particles whatever the di-

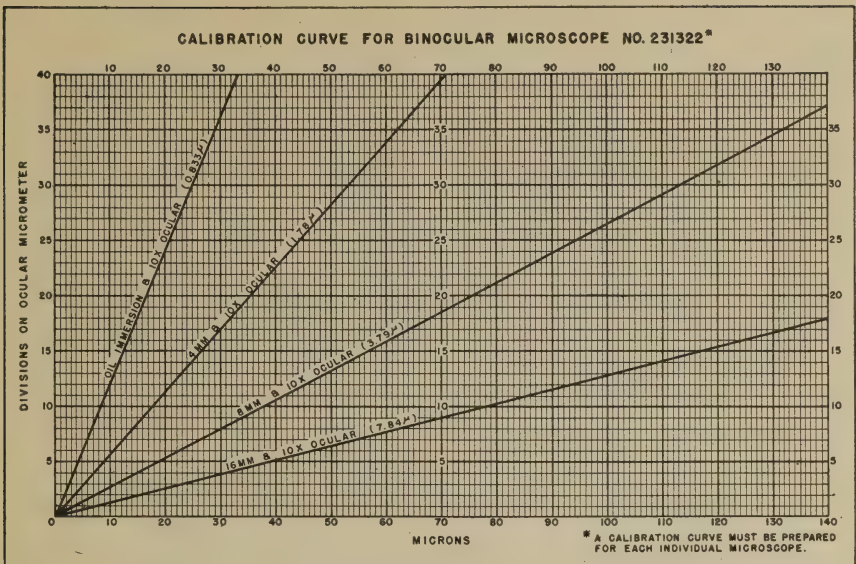


FIG. 14. GRAPH REPRESENTING CALIBRATION OF MICROSCOPE.

lution, or . . . whatever be the size of unit area adopted." Hence, accepting the standard practice of examining ten fields for the field count when the Sedgwick-Rafter method of concentration is used, a series of five fields is sufficient when the centrifuge method is used. This is true because a two liter original sample is used instead of 250 ml. to one liter (usually 500 ml.) amount, and because in addition, the final volume of the concentrate is greater when the Sedgwick-Rafter method is used. The concentration is, therefore, greatest in the average centrifuge sample and many more organisms come within each field that is examined, enough to more than make up for the reduced number of fields examined. The fields selected may be taken at random, but it is suggested that they follow diagonals connecting opposite corners. This method may be used if ten or more organisms oc-

cur in five fields, provided they are present in at least four of these fields. If these conditions are not satisfied, the organisms must be counted as directed in 1.2 or 1.3.

1.2. Strip count. The "strip count" is made by starting the examination at one end of the cell and moving it by means of the mechanical stage toward the other end, proceeding along the longer axis of a  $20 \times 50$  mm. slide. The organisms which are not abundant enough to be counted as in 1.1 are enumerated by this traverse method, in which all forms that occur in a 50 mm. strip 1 mm. deep and the full width of the microscopic field produced by a  $10 \times$  ocular and 8 mm. objective (this will be about 0.7 mm. with ordinary oculars) are included. The use of the 8 mm. objective for this count makes the identification of many forms easier.



# BIOLOGICAL EXAMINATION.—PLANKTON

Location

Serial No.

Date

Concentration

I. to cc.

Factors: Survey to Liter

Strip to 100 L.

Field to 100 L.

Numerator = Number of Organisms.

Denominator = Volume in cubic standard units.

ORGANISMS	Survey Count	Strip Count	FIELD COUNT					Totals	Volume Per 100 L.	Numbers Per 100 L.
			1	2	3	4	5			
I										
II										
III										
IV										
V										
VI										
VII										

Received

Examined

By

(Continued on other side)

FIGS. 15A AND B. SAMPLE FORM OF BENCH SHEET USED WITH CENTRIFUGE METHOD WHEN NUMBERS RATHER THAN CUBIC STANDARD UNITS ARE REPORTED.

1.3. Survey count. The "survey count" consists of enumeration of those organisms which are important yet are so scarce that methods 1.1 and 1.2 are not applicable. It is also applied to the counting of large forms such as *Daphnia*, *Cyclops* and certain rotifers. If the latter forms only are counted, the low-power binocular microscope should be used. If smaller forms are included, the low-power of the compound microscope (16 mm. objective) and the 10 × oculars should be used.

The ideal procedure would be to use the compound microscope for the small forms and the low-power binocular for the larger organisms. Instead of carrying out the "survey count" on the sample used to determine the population of small plankton organisms, the count of larger forms may be made on the concentrate obtained by straining a very large volume of water (50 to 100 liters) through a silk-bolting-cloth type of plankton net.

## 6. Reporting Results in Water Examinations

### A. FORMS

Ruled "bench sheets" should be available to assist in enumeration, calculation and reporting of the organisms that are observed. Two such sample sheets are shown in Fig. 15 and on page 182. Results may be reported in terms of *numbers* of organisms per liter or 100 liters; or as cubic *standard units* per ml., liter or 100 liters.

The form should provide spaces for complete identification of the sample as to place, time and by whom collected and examined. It should show

the concentration and the factors for converting the count into terms of number or volume per liter.

### B. OBSERVATIONS AND REPORTING

Since reporting of numbers is a straightforward procedure, only the method of reporting in terms of cubic standard units is described here. The forms shown in Fig. 15 and on page 182 have been designed to accompany this description.

Obtain a value for the average size of such organisms as occur repeatedly and are fairly uniform in size. The dimensions of the organisms in the view presented under the microscope are measured directly; the third dimension is estimated from a knowledge of the shape of the organisms on the basis of the measured dimension. This value may be in volumetric standard units for spherical, cubical and some irregularly shaped species, or it may be in areal units (the product of two dimensions) for organisms that have a widely varying third dimension. Representatives of species that are not uniform in size must have their bulk estimated as they appear in the survey of total count. The volume of each organism in terms of cubic standard units is obtained as follows:

volume of organism in terms of cubic microns (determined by measurement) ÷ 8000 cubic microns (the volume of one cubic standard unit) = volume of organism in terms of cubic standard units.

Record the value obtained in column (2) of the counting sheet (page 182). If the product of only two dimensions (areal units) is given, designate this.

In the case of species not of uniform size in two or three dimensions, the bulk of which is estimated as seen, there will be no value recorded under column (2) (average size).

Proceed with the survey, and with its completion record in column (3) for each genus or species, showing either the total number of volumetric standard units observed, the number of areal units observed, or the number of individuals seen. A record of the number of individual organisms or cells may be kept simultaneously by noting this number in the counting sheet as the numerator of a fraction, the measurements of the cells being recorded as the denominator, thus,  $4/3$ , four being the number of cells and 3 representing the dimensional values such as volumetric standard units or linear units.

Proceed with the total count for 10 random fields (5 fields, if centrifuge concentration is used). Record in the 10 columns under (4) for each genus or species and for each field, as in the survey, either the total number of volumetric standard units observed, the number of areal units observed, or the number of individuals seen.

A record of the number of individual organisms or cells may be kept simultaneously, again as in the survey, by noting this number in the counting sheet as the numerator of a fraction, the measurements of the cells being recorded as the denominator. When a mechanical stage is employed, the organisms are enumerated as they are covered by the micrometer field, and the observations are entered in the column under (4).

(Note: The computation of volumetric standard units from the individual measurements and estimates is facilitated by the use of a chart (Fig. 16), or by a table such as that given below and commonly included in engineers' and other handbooks.)

A record of amorphous matter in volumetric standard units may also be made for each of the ten fields, or the traverses, examined, and the amount found inserted in the columns under (4) assigned for this purpose.

AREA OF CIRCLE AND VOLUME  
OF SPHERE AND CUBE FOR A  
GIVEN DIAMETER

Diameter.	Area of Circle.	Volume of Sphere.	Volume of Cube.
0.5	0.196	0.065	0.125
1.0	0.785	0.524	1.000
2.0	3.142	4.189	8.000
3.0	7.069	14.137	27.000
4.0	12.566	33.510	64.000
etc., up to 30 or 40			

## C. CALCULATION OF RESULTS

### 1. Cubic Standard Units

The following calculation is used when reporting in cubic standard units and using the Sedgwick-Rafter Method of concentration. (Note that results may also be reported singly as number of organisms by a slight change in this procedure.)

1.1. Record in column (5) the total number of organisms or of units or both, found by examination of the ten fields or by mechanical stage traverses.

1.2. Convert into terms of volumetric standard units the totals recorded for each species under columns (3) and (4). This computation is not necessary where the bulk of a species has been estimated, whenever seen, in terms of volumetric standard units.



1.3. As each value of volumetric units is obtained, convert this into volumetric standard units per ml. of original unconcentrated sample. This

latter value is obtained by multiplying by the survey and total count "multipliers" obtained in the following way.

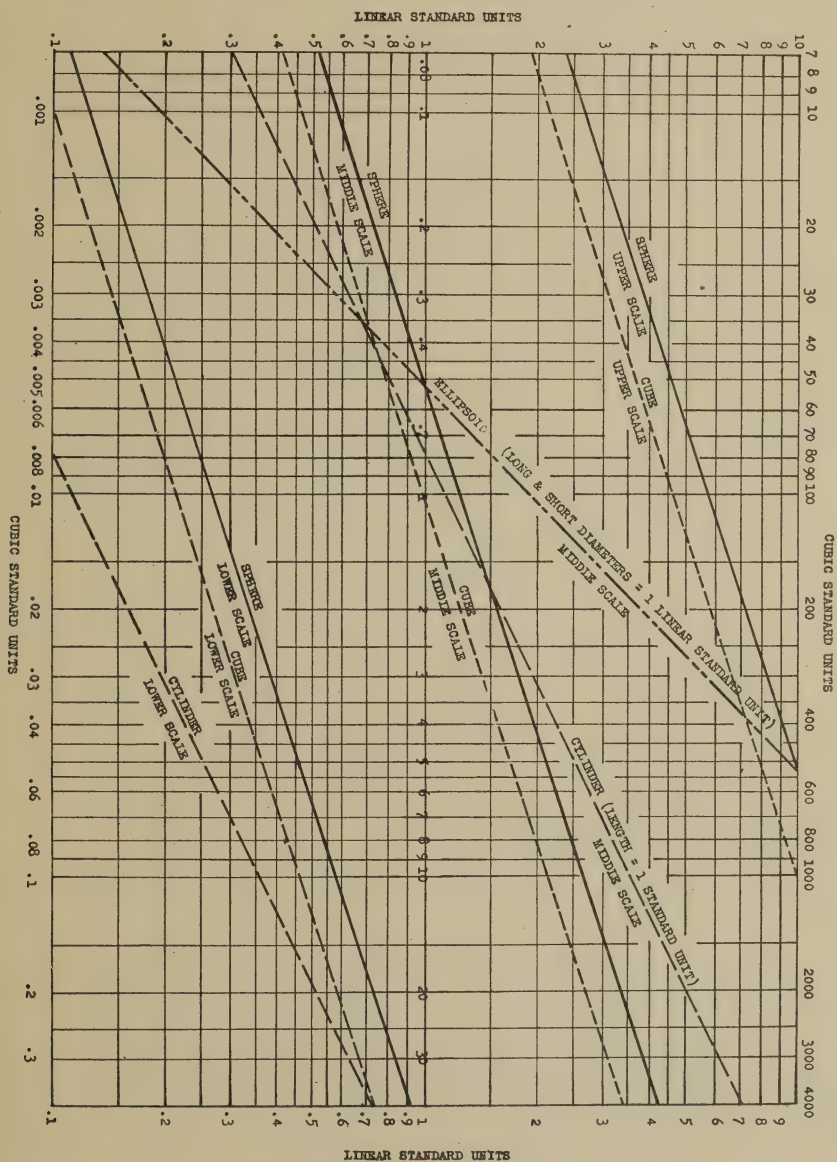


FIG. 16. DETERMINATION OF CUBIC STANDARD UNITS FROM LINEAR DIMENSIONS OF ORGANISMS. From "Microscopy of Drinking Water" by Fair and Whipple, by permission of the publishers, John Wiley & Sons.

$$\left[ \frac{\text{(number of fields in a 1 ml. counting cell 1 mm. deep)}}{\text{(number of fields counted)}} \right] \times \left[ \frac{\text{(ml. of concentrate)}}{\text{(ml. of water filtered)}} \right] = \text{the multiplier.}$$

If, for example, 10 fields are counted in the total count, 250 ml. of water filtered, 15 ml. of distilled water used for the concentrate and the cell holds 1 ml. and is 1 mm. deep, the formula becomes:

$$(1000 \div 10) \times (15 \div 250) = 6, \text{ the total count multiplier.}$$

In the survey the entire cell is examined. If the factors are the same as those above, 1000 fields are covered, and the formula becomes:

$$(1000 \div 1000) \times (15 \div 250) = .06, \text{ the survey multiplier.}$$

Multiplication of the results of the total count and survey by the respective multipliers gives the approximate actual volume of each organism in 1 ml. of water. Moreover, all organisms, large and small, are thus placed on the same definite volumetric basis and their values are comparable. The predominance of any group or species is at once evident.

1.4. Record in columns (6) and (7) the volumetric standard units per ml. of sample for each species observed in the survey and total count. Total the items in each column, then add the two sums to obtain the final result which is expressed as total volumetric standard units or organisms per ml. of sample. The volume of amorphous matter may be computed and recorded in similar fashion. To keep a record of the number of organisms, add to the form two columns headed "Survey" and "Total Count" similar to columns (6) and (7), but

with a main heading "Number of Organisms."

## 2. Number of Organisms

The following calculation is used when reporting in terms of numbers of organisms per unit volume and using the centrifuge method of concentration. (Note that results may also be reported in terms of cubic standard units by a slight change in in procedure.)

2.1. Record "field count" total in column designated for this purpose. This is the total number of organisms found in five fields. (See Fig. 15.)

2.2. Calculate the factors needed to convert the "Survey Count," "Strip Count," and "Field Count" to numbers of organisms per liter of original unconcentrated sample. (Convert to number per 100 liters by multiplying by 100 or to number per ml. by dividing by 1000). This is accomplished in the following way:

2.2.1. Survey count. The entire cell or several cells are examined. If one cell is examined, the factor is obtained as follows:

$$(\text{ml. of concentrate}) \times 1000 \div (\text{ml. of original sample}) = \text{factor.}$$

2.2.2. Strip count. One or several strips may be examined. If one strip is used, then

$$\left[ \frac{\text{(area of cell)}}{\text{(area of strip examined)}} \right] \times \left[ \frac{\text{(ml. of concentrate)}}{\text{(ml. of original sample)}} \right] \times 1000 = \text{factor.}$$

2.2.3. Field count. The total number of organisms in five fields is multiplied by a factor obtained as follows:

$$\left[ \frac{\text{(No. of fields in counting cell)}}{\text{(No. of fields counted)}} \right] \times \left[ \frac{\text{(ml. of concentrate)}}{\text{(ml. of original sample)}} \right] \times 1000 = \text{factor.}$$

## 7. Scope of Examination of Sewage Sludge and Bottom Sediments

The microscopical examination of sewage sludge and bottom sediments may include:

a. The quantitative estimation of the number, bulk or weight of organisms or other material which is retained when the sludge or sediment is sifted through a No. 30 sieve. The material retained may consist of:

1. Large aquatic organisms such as worms, insect larvae and mollusks.

2. Colonial microscopic organisms.

3. Amorphous matter or debris.

b. The quantitative estimation of microscopic organisms or near microscopic organisms which pass a No. 30 sieve but are retained by a No. 100 sieve.

## 8. Apparatus for Sludge Examination

### A. QUANTITATIVE SAMPLES

#### 1. Dredges

A dredge is required for the collection of the sludge or bottom sediment. This dredge should be capable of taking a sample having a known surface area of not less than 36 square inches. Two types of dredges which have been thoroughly tested are now available for this work. One is known as the Ekman dredge and the other as the Petersen dredge. The choice of a dredge is dependent upon the nature of the bottom sediment to be sampled.

#### 2. Sieves

Two screen sieves of standard mesh are needed for the concentration of

the sample. One of these should be a coarse screen, U. S. series equivalent No. 30, having sieve openings of 0.589 mm., the other a U. S. series equivalent No. 100 with sieve openings of 0.149 mm.

#### 3. Containers for sample

A small tub and a three-gallon galvanized pail are needed for the proper manipulation of the sample. Gallon pails, equipped with double friction covers or gallon glass bottles with a wide mouth (four inches or more) and equipped with hard rubber screw caps may be used for the concentrated and preserved samples. Wide-mouth histological bottles of 100 ml. capacity or wide-mouth, one-half pint fruit jars may be used for the sample retained by No. 100 sieve and for special samples.

### B. QUALITATIVE SAMPLES

In some instances, it may be desirable to collect supplementary material not necessarily quantitative. Such samples may be collected by hand, by a dipper or by the equipment listed in A. Other equipment is also the same as in A. Quantitative samples are most valuable, however, and should be collected wherever possible.

### C. ALTERNATIVE APPARATUS

Bottom deposits may be collected by any form of dredge or bucket if its accuracy is as great as that of the apparatus already described. It should be possible to express results obtained by the use of such equipment quantitatively and in the same



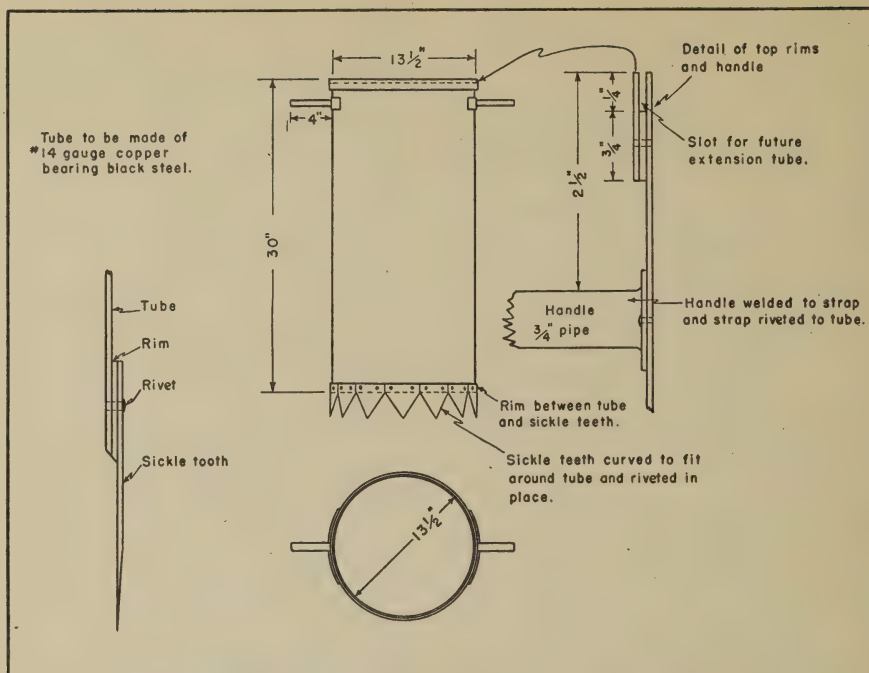


FIG. 17. BOTTOM SAMPLER FOR ROCKY OR PEBBLY BOTTOMS.

manner as if they had been obtained by the recommended procedure.

Where rocky, pebbly bottoms exist and the sampling dredges listed in A are not entirely satisfactory, the bottom sampler shown in Fig. 17 may be used. It consists of a 14-gauge copper-bearing black steel cylinder, 13 1/2 inches in diameter and 30 inches high. The lower end is armed with curved sickle blades which penetrate the deposit when the sampler is forced into it by a rotary motion. Water and rocks as well as the finer sediments are then removed from the cylinder by dipper and by hand and concentrated in the usual manner by straining through the standard sieves. This device can only be used in relatively shallow water, but will be very useful in the smaller streams.

## 9. Collection of Sludge or Sediment Samples

Sampling points shall be carefully selected with a view to obtaining samples which best represent the prevailing conditions.

The dredge is lowered quickly and carefully to the bottom in such a manner that it comes to rest in an upright position on the deposit without scraping loose or disturbing organisms occurring outside the immediate area included in the sample. The sample is then drawn to the surface and emptied into a tub which has been provided for this purpose.

## 10. Concentration of Sludge or Sediment Samples

The first step in concentrating the collected material is to stir the sample

vigorously with a short stick, after which the supernatant water is strained through the No. 30 sieve, having openings of 0.589 mm., and into a 3 gallon pail. The material strained through this sieve is then washed through the finer No. 100 screen having openings of only 0.149 mm.

The sediment recovered in this last straining process is then placed in a 100 ml. histological bottle and is preserved by the addition of commercial formalin to make a 5 to 10 per cent formalin solution. The concentration of the material remaining in the sample after the supernatant water has been removed is the next step. The material is reduced in volume and cleaned by washing in the No. 30 sieve.

The washing process consists of lowering the sieve containing a portion of the sample into the water until it is half submerged. Then the sample is agitated by imparting a swirling motion to the sieve at the same time that it is alternately raised and lowered in the water. This motion tends to turn the sample over and over while a current of water passes alternately up and down through the screen.

The result is a clean, washed concentrate containing a conglomerate collection of animal and vegetable debris, coarse sand or gravel and the organisms which are to be identified and enumerated. The whole sample is treated in this manner, in small batches if necessary, and the residue is placed in a gallon pail where it may be preserved by adding sufficient

10 per cent formalin to cover the material.

If the sample is examined immediately, there is naturally no need for preserving the sample unless the organisms taken are to be kept for a permanent collection. The residue left in the No. 30 sieve in the first step is combined with the concentrate obtained by the washing process.

## 11. Sludge or Sediment

### A. EXAMINATION AND ENUMERATION OF ORGANISMS

If the examination of the sample is made in the laboratory, the sample obtained by the use of the coarse sieve is subjected to another washing process. By this means the formalin fumes, which may be very irritating to the membranes of the nose and eyes, are removed and the organisms are further concentrated for greater convenience in examination.

A strong stream of water is directed into the pail containing the sample until it is filled. Then the supernatant water is quickly poured from the container and strained through the No. 30 sieve. After this process has been repeated several times it will be found that almost all the organisms and most of the vegetable material has been deposited on the screen.

This material is washed into a shallow dish in which it can be spread out in a thin layer. With the aid of a pair of forceps the organisms may then be separated from the debris by hand picking. If the washing has been thorough, very few or no organisms at all will be found in the heavier residue left in the pail.

Because organisms in this residue are very scarce, this part of the sample can be examined very rapidly in spite of its bulk. The treatment of the sample obtained by straining the supernatant water from the freshly collected material through a No. 30 sieve is somewhat different from that given the coarser sample. The organisms retained by the fine No. 100 sieve are much smaller than those retained by the No. 30 sieve.

Because of their minuteness, these organisms must therefore be examined and counted with the aid of a low-power binocular microscope. A magnification of  $20\times$  is convenient, although magnifications ranging from  $5\times$  to  $10\times$  are usually available in this type of instrument.

An aliquot portion of the sample is placed in a Syracuse watch crystal, the bottom of which has been ruled off in parallel guide lines. The distance between the guide lines is slightly less than the field of the microscope and the organisms within the dish may thus be easily enumerated by running the objective over the dish, counting all organisms between the successive lines.

It may be necessary to examine several portions depending upon the volume of the sample. Usually the examination of one-tenth of the sample is sufficient to give adequate information on the organisms contained in it.

## 12. Recording Results of Sludge Examination

The organisms are classified and identified for genus and species where such information is necessary, al-

though incomplete classification may be all that is necessary. The results are expressed in terms of the numbers of organisms of each kind per square yard, and, if so desired, as numbers, volumetric units or grams per liter of sample.

The organisms passing through the coarse sieve and retained by the No. 100 sieve may be recorded on the same basis; e.g., number per square yard and, if so desired, as numbers, volumetric units or grams per liter of sample.

If, for instance, the results are to be expressed in terms of the number of organisms per square yard of bottom area, the number of organisms obtained by washing the sample in a No. 30 sieve is multiplied by a factor which is obtained in the following way:

$$(\text{area of one square yard}) \div (\text{sampling area of dredge}) = \text{factor.}$$

When the sampling area of the dredge is 36 square inches, for instance, the formula becomes:

$$1296 \div 36 = 36, \text{ factor.}$$

In the case of the sample retained by the No. 100 sieve, only a part of the sample is examined critically under the microscope. In order to express the results on the same basis as given above, the calculation is modified as follows:

$$[(\text{total volume of sample}) \div (\text{volume of sample examined})] \times [(\text{area of one square yard}) \div (\text{sampling area of dredge})] = \text{factor.}$$

Where the original volume of the sample is 100 ml. the amount examined, 10 ml., and the sampling area of the



## BIOLOGICAL EXAMINATION OF SEDIMENT

[illegible]

FIG. 18. SAMPLE FORM OF BENCH SHEET USED TO RECORD ORGANISMS FOUND IN  
BOTTOM SEDIMENT SAMPLES.

dredge 36 square inches, the formula becomes :

$100 \div 10 \times 1296 \div 36 = 360$ , the factor.

All results should be carefully recorded and entered on a standard form of the type shown in Fig. 18.

## BIBLIOGRAPHY

- CALKINS, G. N. The microscopical examination of water. *Report State Bd. of Health of Mass.*, 23, 397 (1891).
- CALKINS, G. N. A study of odors observed in drinking water. *Report State Bd. of Health of Mass.*, 355 (1892).
- RAFTER, G. W. *The Microscopical Examination of Water*. New York, 1893.

*nation of Potable Water.* D. Van Nostrand Co., New York (1892).

STUDENT. On the error of counting with a haemocytometer. *Biometrika*, 5, 351 (1907).

WHIPPLE, G. C. Food of oysters. *Report Maryland Board of Shell-Fish Commission*, 324 (1911).

NEEDHAM, J. G., AND LLOYD, J. T. *The Life of Inland Waters*. Comstock Pub. Co., Ithaca, N. Y. (1915).

WARD, H. B., AND WHIPPLE, G. C. *Fresh Water Biology*. J. Wiley & Sons, New York (1918).

PURDY, W. C. Pollution and natural purification of the Ohio River. *Pub. Health Bull. No. 131*, U. S. Pub. Health Service, Washington, D. C. (1922).

JUDAY, C. A third report on Limnological Apparatus. *Trans. Wisconsin Ac. Sc. Arts and Lt., 22*, 299 (1926).

WHIPPLE, G. C., FAIR, G. M., AND WHIPPLE, M. C. *The Microscopy of Drinking Water*. J. Wiley & Sons, New York (1927).

BAYLIS, J. R., AND GERSTEIN, H. H. Microorganisms in Lake Michigan water. *Mun. News and Water Wks.*, 76, 291 (1929).

ABDERHALDEN, EMIL. *Handbuch der biologischen arbeitsmethoden abteilung, 9 Teil 2* (2halfto) Methoden der Susswasserbiologie urband and Schwarzenberg. Berlin. Wien (1936).

OLSON, THEODORE A. Microscopic methods used in biological investigation of lake and stream pollution and interpretation of results. *Sew. Works J.*, 8, 759 (1936).

Form of Report—MICROSCOPIC EXAMINATION.

MICROSCOPICAL EXAMINATION																	
SAMPLE NO.		SOURCE		DATE OF EXAMINATION						EXAMINED BY							
DATE OF COLLECTION										MULTIPLIER:—SURVEY							
CONCENTRATION:—SURVEY				ML. TO ML.						TOTAL COUNT							
				ML. TO ML.													
	Average Size (Stand. Units)	Survey of Cell	NUMBER OF ORGANISMS OR STANDARD UNITS										Volumetric Stand. Units per Ml.		Number of Organisms		
			TOTAL COUNT OF FIELDS											Survey	Total Count	Survey	Total Count
			1	2	3	4	5	6	7	8	9	10	Total				
(1)	(2)	(3)	(4)										(5)	(6)	(7)	(8)	(9)
I. Diatomaceae																	
(1)																	
(2)																	
II. Chlorophyceae																	
(1)																	
(2)																	
(3)																	
III. Cyanophyceae																	
IV. Protozoa																	
(1)																	
(2)																	
V. Rotifera																	
(1)																	
VI. Crustacea																	
(1)																	
VII. Miscellaneous																	
(1)																	
Total Organisms																	
Amorphous Matter																	
Remarks:																	

## PART IV

### BACTERIOLOGICAL EXAMINATION OF WATER

#### 1. Laboratory Apparatus

All laboratory apparatus must be sterile before being used. Glassware, except sample bottles, shall be sterilized for not less than one hour at a temperature of 170° C., unless it is known by means of recording thermometers that the oven temperatures are uniform, under which exceptional condition 160° C. may be used.

Sample bottles may be sterilized as above or may be sterilized in a steam autoclave at 15 lb. pressure for one-half hour.

##### A. SAMPLE BOTTLES

A glass bottle of good quality of any size or shape may be used for the bacterial sample provided it holds a sufficient amount for all of the tests required, and is such that it may be properly washed and sterilized and will keep the sample uncontaminated until the analysis is made. Ground-glass stoppered bottles (preferably wide mouth) of good quality glass are recommended. The stopper and neck should be covered with metal foil or suitable paper substitute (milk bottle caps, etc.), and the bottle packed in a dust-proof package, for transportation.

##### B. PIPETTES

Pipettes may be of any convenient size or shape provided it is found by actual test that they deliver accu-

rately the required amount in the manner in which they are used. The error of calibration shall in no case exceed 2 per cent.

It is recommended that the mouth end of all pipettes be protected with a cotton plug.

##### C. DILUTION BOTTLES

Bottles for use in making dilutions should preferably be of tall form and of such capacity as to hold at least twice the volume of water actually used. Close fitting ground-glass or rubber stoppers should be used.

Dilution bottles as recommended in *Standard Methods for the Examination of Dairy Products* may be used.

##### D. PETRI DISHES

Petri dishes 10 cm. in diameter with the side wall of the bottom at least 1 cm. high shall be used with glass or porous tops as preferred. The bottoms of the dishes shall be free from bubbles and as flat as possible so that the medium shall be of uniform thickness throughout the plate.

##### E. FERMENTATION TUBES

A fermentation tube of any type may be used provided it permits conformance to the requirement for concentration of nutritive ingredients. (Part IV, Sec. 3, N, 2, page 188, and Sec. 9, B, 1, page 194.)



## BIBLIOGRAPHY

- HILL, H. W. Porous tops for Petri dishes. *J. Med. Res.*, 13, 93 (1904).
- BROWNE, W. W. A comparative study of the Smith fermentation tube and the inverted vial in the determination of sugar fermentation. *Am. J. Pub. Health*, 3, 701 (1913).
- COLLINS, W. D., AND RIFFENBURG, H. B. Contamination of water samples with material dissolved from glass containers. *Ind. Eng. Chem.*, 15, 48 (1923).

## 2. Materials

## A. WATER

Distilled water shall be used in the preparation of all culture media and reagents.

## B. MEAT EXTRACT

Bacto-beef extract, or any other brand giving equivalent results, shall be used. Meat infusion shall not be used.

## C. PEPTONE

Bacto-peptone, or any other peptone which comparative tests have shown to give equivalent results, may be used.

## D. SUGARS

All sugars used shall be of the highest purity.

## E. AGAR

The agar used shall be of the best quality. If not specially prepared for bacteriological work, agar should be soaked in distilled water and drained before use.

## F. GELATIN

The gelatin used shall be of light color, shall not contain objectionable impurities, and shall be free from

preservatives. The melting point shall be such that a 10 per cent standard nutrient gelatin shall melt at 25° C. or over.

## G. GENERAL CHEMICALS

Special effort shall be made to have all the ingredients used for culture media chemically pure.

## H. DYES

Dyes certified by the Commission on Standardization of Biological Stains for use in the preparation of media shall be employed.

## BIBLIOGRAPHY

- SMITH, H. M. The seaweed industries of Japan. *Bull. of the Bureau of Fisheries*, 24, 135 (1904).
- WHITTAKER, H. A. The source, manufacture and composition of commercial agar-agar. *Am. J. Pub. Health*, 1, 632 (1911).
- NOYES, H. A. Agar-agar for bacteriological use. *Science*, n. s., 44, 797 (1916).

## 3. Preparation of Culture Media

## A. ADJUSTMENT OF REACTION

The reaction of culture media shall be stated in terms of hydrogen-ion concentration, expressed as pH values.

The increase in the hydrogen-ion concentration (decrease in pH) during sterilization will vary slightly with the individual sterilizer in use and the initial reaction required to obtain the correct final reaction will have to be determined. The decrease in the pH reading will usually be between 0.2 and 0.4.

## 1. Procedure

1.1. Electrometric method. Tests to control the adjustment to the re-

quired hydrogen-ion concentration should, preferably, be made electrometrically (see Part I, Sec. 11, A, page 28).

1.2. Colorimetric method. Add 5 ml. of distilled water to each of two clean test tubes, similar in size, shape and color to the tubes used for the color standards—6 inch by  $\frac{1}{2}$  inch tubes are recommended. Withdraw 10 ml. of the medium to be adjusted and add 5 ml. to each of the two tubes. To one of these add 0.5 ml. of a solution of an indicator which will adequately cover the desired pH range. The amount of indicator in the test solution must be the same as in the standard. This is usually 0.5 ml. (For preparation, see Part I, Sec. 11, B, page 30.)

Using a comparator block, superimpose the tube containing the diluted medium plus the indicator over a tube of distilled water and superimpose the tube of diluted medium without indicator over the color standards of the pH desired.

Titrate the tube of diluted medium plus indicator with an accurate 1:10 dilution of an approximately normal NaOH soln. until the color viewed through the distilled water tube matches the color of the pH standard as observed through the diluted medium without the indicator.

Calculate the amount of N NaOH soln. which must be added to the medium to reach this reaction. After the addition and thorough mixing, check the reaction. The required final reaction is given in the directions for preparing each medium. If specific reaction is not described, adjustment is not necessary.

## B. STERILIZATION

All media, except as directed for the preparation of sugar broths, shall be sterilized in the autoclave at 15 lb. ( $121^{\circ}$  C.) for 15 minutes after the pressure has reached 15 lb. All air must be forced out of the autoclave by allowing live steam to stream through it for a few minutes before the pressure is allowed to rise. As soon as possible after sterilization the medium shall be removed from the autoclave and cooled quickly. Rapid and immediate cooling of gelatin and lactose media is imperative.

Media shall be sterilized in small containers and these must not be closely packed together. No part of the medium shall be more than 2.5 cm. from either the outside surface of the glass or the surface of the medium.

## C. CLARIFICATION

The extent to which a medium shall be clarified depends on the use to which the medium is put and will vary also according to the experience and preference of the laboratory worker. It is permissible to use any method of clarification which will yield a medium sufficiently clear for detection of bacterial growth and which will not add or remove necessary nutritive ingredients. Methods may include clarification in a centrifuge, or filtration through paper, cotton, cheese cloth, or towels.

## D. NUTRIENT BROTH

Add 3 g. of beef extract and 5 g. of peptone to 1000 ml. of distilled water. Heat slowly on a water bath to  $65^{\circ}$  C., stirring until dissolved,

Make up lost weight with distilled water. Adjust the reaction so that the pH reading after sterilization will be between 6.4 and 7.0.

Bring to a boil over a free flame, cool to 25° C., make up lost weight with distilled water and clarify as desired. Distribute in test tubes, 10 ml. to each tube, or in other desired containers. Sterilize as directed under Part IV, Sec. 3, B, page 185.

#### E. LACTOSE BROTH

To nutrient broth as prepared in Part IV, Sec. 3, D, add 0.5 per cent of lactose.

Adjust the reaction so that the pH reading after sterilization will be between 6.4–7.0, preferably 6.9. Place in fermentation tubes and sterilize as directed under Part IV, Sec. 3, B, page 185, provided that the total time of exposure to any heat is not more than one-half hour. Cool rapidly after removal from the autoclave.

If the above condition of exposure to heat cannot be fulfilled, prepare a 10 or 20 per cent solution of lactose in distilled water and sterilize as directed under Part IV, Sec. 3, B, page 185, or by heating in an Arnold sterilizer at 100° C. for 1½ hours. Add this solution to sterile nutrient broth in amount sufficient to make a 0.5 per cent lactose solution, tube with proper precautions for preserving its sterility, and sterilize in flowing steam at 100° C. for 30 minutes.

It is permissible to add, by means of a sterile pipette, directly to a tube of sterile nutrient broth, enough of the lactose solution to make the required 0.5 per cent concentration. The tubes so prepared shall be incu-

bated at 37° C. for 24 hours as a test for sterility, before they are used.

#### F. NUTRIENT GELATIN

Add 3 g. of beef extract, 5 g. of peptone, and 120 g. of gelatin (undried market product as stored in the ordinary laboratory cupboard) to 1 liter of distilled water.

Heat slowly on a water bath to 65° C. until all ingredients are dissolved.

Make up lost weight with distilled water and adjust the reaction so that the pH reading after sterilization will be between 6.4 and 7.0.

Bring to a boil, stirring vigorously. Make up lost weight with distilled water and clarify.

Distribute to the desired containers and sterilize as directed under Part IV, Sec. 3, B, page 185.

#### G. NUTRIENT AGAR

Add 3 g. of beef extract, 5 g. of peptone and 15 g. of agar (undried market product as stored in the ordinary laboratory cupboard) to 1 liter of distilled water. Boil until all the agar is dissolved. Make up lost weight with hot distilled water. Adjust the reaction so that the pH reading after sterilization will be between 6.4 and 7.0.

Bring to a boiling temperature, with frequent stirring, restore lost weight with hot distilled water, and clarify.

Distribute in the desired containers and sterilize as directed under Part IV, Sec. 3, B, page 185.

#### H. TRYPTONE GLUCOSE EXTRACT AGAR

To 1 liter of distilled water, add 3 g. of beef extract, 5 g. of tryptone,



1 g. of glucose and 15 g. of agar (undried market product as purchased). Boil until all ingredients are dissolved. Make up lost weight with hot distilled water. Adjust the reaction so that the pH reading after sterilization will be between 6.6 and 7.0.

Bring to a boiling temperature, stirring vigorously. Make up lost weight with hot distilled water and clarify.

Distribute to the desired containers and sterilize as directed under Part IV, Sec. 3, B, page 185.

## I. ENDO MEDIUM

### 1. Formula I

1.1. Preparation of stock agar. Add 5 g. of beef extract, 10 g. of peptone and 30 g. of agar to 1 liter of distilled water (undried market product as stored in the ordinary laboratory cupboard).

Boil until the agar is dissolved and make up lost weight with distilled water.

Adjust the reaction so that the pH reading after sterilization will be 7.4.

Clarify if desired.

Add 10 g. of lactose and dissolve.

Place in small flasks or bottles, 100 ml. to each, and sterilize in autoclave as directed under Part IV, Sec. 3, B, page 185.

1.2. Preparation of plates. Prepare a 3 per cent solution of certified basic fuchsin in 95 per cent ethyl alcohol.

Allow to stand 24 hours and filter.

Melt lactose agar as prepared above and to each 100 ml. add 1 ml. of the 3 per cent basic fuchsin solution and 0.125 g. of anhydrous sodium sulfite dissolved in 5 ml. of distilled water.

The sulfite solution must be freshly prepared.

Mix thoroughly, pour plates with usual precautions against contamination and allow to harden.

The medium should be light pink when hot and almost colorless when cool. As batches of fuchsin differ somewhat in dye content, it is possible that the medium made up according to this formula may be too highly colored before incubation or may not give the proper reaction when seeded with coliform organisms. In such case, the strength of the basic fuchsin solution may be varied.

### 2. FORMULA II

An alternative to the directions given above is the following formula:

Dipotassium phosphate $K_2HPO_4$	3.5	g.
Bacto peptone	10.0	g.
Agar (washed and dried)	15.0	g.
Water (distilled)	1.0	l.
Lactose	10.0	g.

To each 100 ml. of the above add:

Sodium sulfite (anhydrous)	0.25	g.
Basic fuchsin (pararosanilin), filtered 10 per cent alcoholic solution*	0.5	ml.

\* Some basic fuchsin is not soluble enough to make a 10 per cent alcoholic solution.

## J. EOSIN METHYLENE-BLUE AGAR

Add 10 g. of peptone, 2 g. of dipotassium phosphate ( $K_2HPO_4$ ) and 15 g. of undried agar to 1 liter of distilled water.

Boil until all ingredients are dissolved and make up loss due to evaporation with distilled water.

Adjustment of reaction is not necessary.

Place measured quantities (100 or 200 ml.) in flasks or bottles and sterilize in the autoclave as directed under Part IV, Sec. 3, B, page 185.

To prepare plates, melt stock agar as described above and to each 100 ml. add 5 ml. of sterile 20 per cent aqueous lactose solution, 2 ml. of 2 per cent aqueous solution of eosin, yellowish, and 1.3 ml. of 0.5 per cent aqueous solution of methylene-blue.

Mix thoroughly, pour into Petri dishes and allow to harden.

It is permissible to add all of the ingredients to the stock agar at the time of preparation, place in tubes or flasks, and sterilize. Plates may be prepared from this stock. Decolorization of the medium occurs during sterilization, but the color returns after cooling.

#### K. BRILLIANT-GREEN LACTOSE BILE BROTH

Dissolve 10 g. of peptone and 10 g. of lactose in not more than 500 ml. of distilled water.

Add 200 ml. of fresh ox bile, or 20 g. of dehydrated oxgall dissolved in 200 ml. of distilled water. The solution of dehydrated oxgall shall have a pH between 7.0 and 7.5.

Make up with distilled water to approximately 975 ml.

Adjust the reaction to a pH reading of 7.4.

Add 13.3 ml. of a 0.1 per cent solution of brilliant green in distilled water.

Add sufficient distilled water to make the volume 1 liter.

Filter through cotton.

Distribute in fermentation tubes and sterilize as directed under Part IV, Sec. 3, E, page 186.

The reaction after sterilization (determined by potentiometric and not by colorimetric methods) should

be not less than pH 7.1 and not more than pH 7.4.

#### L. FORMATE RICINOLEATE BROTH

Add 5 g. of peptone, 5 g. of lactose, 5 g. of sodium formate and 1 g. of sodium ricinoleate to 1 liter of distilled water.

Heat slowly on a water bath with constant stirring, until dissolved.

Add distilled water to make the volume 1 liter.

Adjust the reaction so that the pH reading after sterilization will be 7.3 to 7.5.

Distribute in fermentation tubes and autoclave at 11 to 13 lb. pressure for 15 minutes.

#### M. LAURYL SULFATE TRYPTOSE BROTH

Add 20 g. of tryptose, 5 g. of lactose, 2.75 g. of dibasic potassium phosphate, 2.75 g. of monobasic potassium phosphate, 5 g. of sodium chloride and 0.1 g. Duponol W A Flakes\* (sodium lauryl sulfate) to 1 liter cold distilled water. Dissolve ingredients, distribute into fermentation tubes and autoclave as directed in Part IV, Sec. 3, E, page 186. The final pH should be approximately 6.8.

#### N. PERMISSIBLE VARIATIONS IN MEDIA

##### 1. *Dehydrated Media*

The use of dehydrated media is permissible provided comparative tests have shown that these media give results equivalent to those secured with freshly prepared media.

##### 2. *Concentration of Ingredients*

The concentrations of the ingredients in the final mixture of medium

\* Manufactured by E. I. DuPont de Nemours & Co., Wilmington, Delaware. Also supplied by Canadian Industries Limited, Montreal, Canada.

and sample in fermentation tubes containing lactose broth or lauryl sulfate tryptose broth must be maintained as indicated in the formulae specified for these media. For example, 10 ml. of water sample may be added to 10 ml. of double strength lactose broth, and 100 ml. of sample may be added to 50 ml. of triple strength lactose broth (although 1 ml. or less of sample may be added to 10 ml. of the single strength broth).

#### O. STORAGE OF CULTURE MEDIA

Culture media should be stored at refrigeration temperature in order to prevent excessive evaporation, unless they are to be used within a short time after preparation.

Liquid media in fermentation tubes, however, if stored at refrigeration or even at moderately low temperatures, may dissolve sufficient air to produce, upon incubation at 37° C., a bubble of gas in the tube. It is imperative, therefore, that fermentation tubes which have been at a low temperature be incubated overnight before use and those tubes containing gas discarded.

Fermentation tubes may be stored at approximately 25° C.; but since evaporation may proceed rapidly under these conditions, resulting in marked changes in concentration of the ingredients, storage at this temperature should not exceed a period of one week.

#### BIBLIOGRAPHY

ENDO, S. On a method for the detection of the typhoid bacillus. *Centrbl. f. Bakt., Abt. I*, 35, 109 (1903-4).  
KENDALL, A. I., AND WALKER, A. W. The isolation of *Bacillus dysenteriae* from stools. *J. Med. Res.*, 23, 481 (1910).

KINYOUN, J. J., AND DEITER, L. V. On the preparation of Endo's medium. *Am. J. Pub. Health*, 2, 979 (1912).  
CLARK, W. M., AND LUBS, H. A. On some new indicators for the colorimetric determination of hydrogen-ion concentration. *J. Wash. Acad. Sciences*, 5, 609 (1915).  
MCCLENDON, J. F. A direct reading potentiometer for measuring hydrogen-ion concentrations. *Am. J. Physiol.*, 38, 186 (1915).  
CLARK, W. M. The "reaction" of bacteriologic culture media. *J. Infect. Dis.*, 17, 109 (1915).  
CLARK, W. M., AND LUBS, H. A. Hydrogen electrode potentials of phthalate, phosphate, and borate buffer mixtures. *J. Biol. Chem.*, 25, 479 (1916).  
CLARK, W. M., AND LUBS, H. A. The colorimetric determination of hydrogen-ion concentration and its application in bacteriology. *J. Bact.*, 2, 1 and 109 (1917).  
MUEER, T. C., AND HARRIS, R. L. Value of brilliant green in eliminating errors due to the anaerobes in the presumptive test for *B. coli*. *Am. J. Public Health*, 10, 874 (1920).  
LEVINE, MAX. Bacteria fermentating lactose and their significance in water analysis. *Iowa State College of Agr. and Mech. Arts Bull.*, 62, 117 (1921).  
BUNKER, G. C., AND SCHUBER, H. The reaction of culture media. *J. Amer. W. W. Assn.*, 9, 63 (1922).  
WINSLOW, C.-E. A., AND DOLLOFF, A. F. The relative effect of certain triphenylmethane dyes upon the growth of bacilli of the colon group in lactose broth and lactose bile. *J. Infect. Dis.*, 31, 302 (1922).  
HARRIS, N. MACL. The preparation of Endo's medium. *Mil. Surgeon*, 57, 280 (1925).  
DUNHAM, H. G., MCCRADY, M. H., AND JORDAN, H. E. Studies of differential media for detection of *Bacterium coli* in water. *J. Amer. W. W. Assn.*, 14, 535 (1925).  
JORDAN, H. E. Brilliant green bile for the detection of the coli-aerogenes group. *J. Amer. W. W. Assn.*, 18, 337 (1927).  
JORDAN, H. E. Brilliant green bile for coli-aerogenes group determinations. *J. Amer. W. W. Assn.*, 24, 1027 (1932).  
CONN, H. J., AND DARROW, MARY A. Can the Endo medium be standardized? *Stain Technology*, IX, 2, 61 (1934).



- RUCHHOFT, C. C. Comparative studies of media for the determination of the coli-aerogenes group in water analysis. *J. Amer. W. W. Assn.*, 27, 1732 (1935).
- BOWERS, C. S., AND HUCKER, G. J. The composition of media in the bacteriological analysis of milk. *New York Agr. Exp. Sta. Tech. Bull.*, 223 (1935).
- RUCHHOFT, C. C., AND NORTON, J. F. Study of selective media for coli-aerogenes isolations. *J. Amer. W. W. Assn.*, 27, 1134 (1935).
- STARK, C. N., AND ENGLAND, C. W. Formate ricinoleate broth—a new medium for the detection of colon organisms in water and milk. *J. Bact.*, 29, 26 (1935).
- MCCRADY, M. H. A practical study of procedures for the detection of the presence of coliform organisms in water. *Am. J. Pub. Health*, 27, 1243 (1937).
- COWLES, P. B. A modified lactose broth for use in the presumptive test. *J. Amer. W. W. Assn.*, 30, 979 (1938).
- MCCRADY, M. H. A comparison of MacConkey's broth and standard lactose broth as media for detection of coliform organisms in water. *Am. J. Pub. Health*, 29, 1250 (1939).
- DARBY, C. W., AND MALLMANN, W. L. Studies on media for coliform organisms. *J. Amer. W. W. Assn.*, 31, 689 (1939).
- KELLEY, C. E. Brilliant green lactose bile and the Standard Methods Completed Test in isolation of coliform organisms. *Am. J. Pub. Health*, 30, 1034 (1940).
- TAYLOR, D. M. A study of procedures for detection of coliform organisms in Minnesota water. *J. Amer. W. W. Assn.*, 32, 98 (1940).
- RICHEY, DALE. Relative value of 2 per cent and 5 per cent brilliant green bile confirmatory media. *J. Amer. W. W. Assn.*, 33, 649 (1941).
- HOWARD, N. J., LOCHHEAD, A. G., AND MCCRADY, M. H. A study of methods for the detection of the presence of coliform organisms in water. *Can. Pub. Health J.*, 32, 29 (1941).
- MALLMANN, W. L., AND DARBY, C. W. Uses of a lauryl sulphate tryptose broth for the detection of coliform organisms. *Am. J. Pub. Health*, 31, 127 (1941).
- MALLMANN, W. L., AND BREED, R. S. A comparative study of standard agars for determining bacterial counts in water. *Am. J. Pub. Health*, 31, 341 (1941).
- HOWARD, N. J., LOCHHEAD, A. G., AND MCCRADY, M. H. Report of the Committee on Bacteriological Examination of Water and Sewage. *Can. Pub. Health J.*, 33, 49 (1942).
- ARCHAMBAULT, J., AND MCCRADY, M. H. Dissolved air as a source of error in fermentation tube results. *Am. J. Pub. Health*, 32, 1164 (1942).
- WATTIE, ELSIE. Coliform confirmation from raw and chlorinated waters with brilliant green bile lactose broth. *Pub. Health Repts.*, 53, 377 (1943).
- MCCRADY, M. H. A practical study of lauryl sulphate tryptose broth for detection of the presence of coliform organisms in water. *Am. J. Pub. Health*, 33, 1199 (1943).
- LEVINE, MAX. The effect of concentration of dyes on differentiation of enteric bacteria on eosin-methylene-blue agar. *J. Bact.*, 45, 471 (1944).

#### 4. Samples

##### A. COLLECTION

Samples for bacterial analysis shall be collected in bottles which have been cleansed with great care, rinsed in clean water, and sterilized as directed under Part IV, Sec. 1, page 183.

Bottles destined for collection of waters containing residual chlorine (swimming pool, treated, etc.), unless they contain broth for direct planting of sample therein (Part IV, Sec. 9, B, 1, page 194), should be prepared as follows:

Into clean, dry bottles is placed from 0.02 to 0.05 g. powdered sodium thiosulfate. The bottles are then stoppered and capped as directed under Part IV, Sec. 1, A, page 183, and sterilized by dry heat as directed under Part IV, Sec. 1, page 183.

Great care must be exercised to have the samples representative of the water to be tested and to see that no contamination occurs at the time of filling the bottles or prior to examination. Ample air space should be left between the stopper and the

level of the water sample in the bottle in order to facilitate mixing of the sample by shaking, preparatory to examination.

#### B. STORAGE AND TRANSPORTATION

Because of the rapid and often extensive changes which may take place in the bacterial flora of bottled samples when stored even at temperatures as low as 10° C., it is urged, as a matter of importance, that all samples be examined as promptly as possible after collection.

The time allowed for storage or transportation of a bacterial sample between the filling of the sample bottle and the beginning of the analysis should not be more than six hours for impure waters and not more than twelve hours for relatively pure waters. During the period of storage, the temperature shall be kept between 6° C. and 10° C. Any deviation from the above limits shall be so stated in making reports.

#### 5. Dilutions

Dilution bottles shall be sterilized in the autoclave at 15 lb. (121° C.) for 15 minutes after the pressure reaches 15 lb.

Dilution bottles or tubes shall be filled with the proper amount of water so that after sterilization they shall contain 9 ml. or 99 ml., as desired, with a tolerance of 2 per cent. The exact amount of water to be placed in the bottles may be determined only by experiment with the particular autoclave in use. If desired, the 9 ml. dilution may be measured from a flask of sterile water with a sterile pipette.

The water used for dilution shall be tap water, or the phosphate dilution water recommended in the procedure for biochemical oxygen demand (Part II, Sec. 11, A, 2.1, page 140). Distilled water shall not be used.

The sample bottle shall be shaken vigorously 25 times and 1 ml. withdrawn and added to the proper dilution bottle or tube as required. Each dilution bottle or tube after the addition of the 1 ml. of the sample shall be shaken vigorously 25 times before a second dilution is made from it, or before a portion is removed.

#### BIBLIOGRAPHY

BUTTERFIELD, C. T. The selection of dilution waters for bacteriological examinations. *Pub. Health Repts.* (Rept. 1580), 43, 681 (1933).

#### 6. Plating

Plating shall be completed within 20 minutes after dilutions are made. One-half ml. or one ml. of the sample or dilution (see Sec. 8) shall be used for plating and shall be placed in the Petri dish first. Ten ml. of liquefied medium (nutrient agar, tryptone glucose extract agar,\* or gelatin) at a temperature of about 42° C. shall be added to the water in the Petri dish.

The cover of the Petri dish shall be lifted just enough for the introduction of either the pipette or culture medium and the lips of all test tubes or flasks used for pouring the medium shall be flamed. The medium and sample in the Petri dish shall be thor-

\* The option of using tryptone glucose agar is offered so that laboratories that plate both milk and water samples need not stock two different agars for this purpose. Available experimental data indicate that this agar and nutrient agar give practically the same counts with water samples, and that colonies are larger and more easily counted when tryptone glucose agar is employed.

oughly mixed and uniformly spread over the bottom of the Petri dish by tilting and rotating the dish. All plates shall be solidified as rapidly as possible after pouring and placed immediately in the appropriate incubator.

#### BIBLIOGRAPHY

BOWERS, C. S., AND HUCKER, G. J. The composition of media in the bacteriological analysis of milk. *New York Agr. Exp. Sta. Tech. Bull.*, 228 (1935).

MALLMANN, W. L., AND BREED, R. S. A comparative study of standard agars for determining bacterial counts in water. *Am. J. Pub. Health*, 31, 341 (1941).

HOWARD, N. J., LOCHHEAD, A. G., AND McCRADY, M. H. Report of Committee on Bacteriological Examination of Water and Sewage. *Can. Pub. Health J.*, 33, 49 (1942).

#### 7. Incubation

Gelatin plates shall be incubated for  $48 \pm 3$  hours at  $19^{\circ}$  C. to  $21^{\circ}$  C. in a dark, well-ventilated incubator in an atmosphere practically saturated with moisture.

Agar plates may be used for counts made either at  $19^{\circ}$  C. to  $21^{\circ}$  C. or  $35^{\circ}$  C. to  $37^{\circ}$  C. The time for incubation at the lower temperature shall be  $48 \pm 3$  hours and that at the higher temperature,  $24 \pm 2$  hours. The incubators shall be dark, well ventilated and the atmosphere shall be practically saturated with moisture. Glass covered plates shall be inverted in the incubator. Any deviation from the above described method shall be stated in making reports. Plates shall not be closely packed.

When reporting the results of water examination, the medium used for the total count should be stated, i.e., whether gelatin or agar, and the temperature of incubation given.

#### BIBLIOGRAPHY

WHIPPLE, G. C. On the necessity of cultivating water bacteria in an atmosphere saturated with moisture. *Technology Quart. (M. I. T.)*, 12, 276 (1899).

*Standard Methods for the Examination of Dairy Products.* Am. Pub. Health Assn., 8th Ed., p. 29 (1941).

#### 8. Counting

In preparing plates, such amounts of the water under examination shall be planted as will give from 30 to 300 colonies on a plate and the aim should be always to have at least two plates giving colonies between these limits.

Where it is possible to obtain plates showing density of colonies within these limits, only such plates should be considered in recording results, except when the same amount of water has been planted in two or more plates, of which one gives colonies within these limits, while others give less than 30 or more than 300. In such case, the result recorded should be the average of all the plates planted with this amount of water.

Ordinarily, it is not desirable to plant more than 1 ml. of water in a plate; therefore, when the total number of colonies developing from 1 ml. is less than 30, it is obviously necessary to record the result as observed, disregarding the general rule given above.

Counting shall be done with a lens giving a magnification of  $11\frac{1}{2}$  diameters. An approved counting aid, known as the Quebec Colony Counter is recommended. In order to insure uniformity of counting conditions, illumination equivalent to that provided by the Quebec Colony Counter shall be employed.



In order to avoid fictitious accuracy and yet express the numerical results by a method consistent with the precision of the technique employed, the recorded number of bacteria per ml. shall include not more than two significant figures. For example, a count of 142 is recorded as 140, and a count of 145 is recorded as 150; whereas a count of 35 is recorded as such.

The gelatin count at 19° C. to 21° C. and the agar counts at 19° C. to 21° C. and at 35° C. to 37° C. shall be designated "standard gelatin plate count," "20° C. standard agar plate count," and "37° C. standard agar plate count," respectively.

#### BIBLIOGRAPHY

- BREED, R. S., AND DOTTERER, W. D. The number of colonies allowable on satisfactory agar plates. *J. Bact.*, 1, 321 (1916).  
ARCHAMBAULT, J., CUROT, J., AND MCCRADY, M. H. The need of uniformity of conditions for counting plates. *Am. J. Pub. Health*, 27, 809 (1937).  
JENNISON, MARSHALL W., AND WADSWORTH, GEORGE P. Evaluation of the errors involved in estimating bacterial numbers by the plating method. *J. Bact.*, 39, 389 (1940).

### 9. Tests for the Presence of Members of the Coliform Group

#### A. INTRODUCTION AND DEFINITIONS

##### 1. Definition

It is recommended that the coliform group be considered to include all aerobic and facultative anaerobic Gram-negative non-spore-forming bacilli which ferment lactose with gas formation.

The "coliform group" as defined above is equivalent to the "B. coli group" as used in all editions of

*Standard Methods for the Examination of Water and Sewage* prior to the sixth edition, and to the "coli-aerogenes group" of later editions.

##### 2. The Standard Tests

The standard tests for the coliform group shall be either the Presumptive Test, the Confirmed Test, or the Completed Test as hereinafter defined, each test being applicable under the circumstances specified in Sec. 9, F, below.

In these standard tests lauryl sulfate tryptose broth may be substituted for lactose broth in the examination of all waters except final filtered, treated, and filtered-treated waters. It may be substituted for lactose broth also in the examination of final filtered, treated and filtered-treated waters provided the laboratory worker has amply demonstrated by correlation of positive completed tests (isolations of coliform organisms) secured through the use of lauryl sulfate tryptose broth with those secured through the use of lactose broth, in the examination of such waters, that the substitution results in no reduction from the density of coliform organisms indicated by the standard procedure using lactose broth.

##### 3. Presumptive Test

The formation of gas in a standard lactose broth fermentation tube at any time within  $24 \pm 2$  hours with incubation at 35° C. to 37° C. is presumptive evidence of the presence of coliform organisms, since the majority of the bacilli which give such a reaction belong to the group.

#### 4. *Confirmed Test*

The formation of gas at any time within  $48 \pm 3$  hours with incubation at  $35^{\circ}\text{C}$ . to  $37^{\circ}\text{C}$ . in a fermentation tube containing brilliant green lactose bile broth which has been seeded from a lactose broth fermentation tube in which gas has formed, or the appearance of aerobic lactose-splitting, typical *Escherichia coli* colonies on a specified solid confirmatory medium streaked from a lactose fermentation tube in which gas has formed, confirms the presumption that gas formation in the fermentation tube was due to the presence of members of the coliform group. (If only colonies *not* typical of *Escherichia coli* have developed on the solid medium, the Completed Test is to be applied.)

For an alternate medium, see Part IV, Sec. 9, C, page 195.

#### 5. *Completed Test*

To complete the demonstration of the presence of organisms of this group, it is necessary to show that one or more aerobic plate colonies consist of Gram-negative non-spore-forming bacilli, which, when inoculated into a lactose broth fermentation tube, form gas.

#### 6. *Reporting Results*

In reporting results, the particular test (Presumptive, Confirmed or Completed) applied to the sample should be recorded.

#### 7. *Differentiation*

When it is desired to differentiate between the various sections of the coliform group, the detailed procedure shall not follow primary plant-

ing in liquid media, but shall be based upon primary planting of the sample in solid media. (See Appendix I, Sec. 13, page 227 and Sec. 14, page 228.)

### B. PRESUMPTIVE TEST

#### 1. *Procedure*

Inoculate a series of lactose broth fermentation tubes with appropriate graduated quantities of the water to be tested. The concentration of nutritive ingredients in the mixture of medium and added portion of sample must conform to the requirements given under Part IV, Sec. 3, N, 2, page 188. The portions of the water sample used for inoculating the lactose broth fermentation tubes will necessarily vary in size with the character of the water under examination (see Part IV, Sec. 11, B, C, D, pages 202-3).

Comparatively large volumes (e.g. 100 ml.) of sample, intended for detection of the presence of coliform organisms, may be planted directly into lactose broth at the site of collection of the sample, using ordinary dilution bottles, six ounce or eight ounce bottles, containing multiple-strength lactose broth and equipped with inverted vial or Cowles tube. Such bottles may be marked with graduations to eliminate the necessity of using pipettes for transfer of medium and sample.

Incubate the fermentation tubes at  $35^{\circ}\text{C}$ . to  $37^{\circ}\text{C}$ . for  $48 \pm 3$  hours unless gas appears earlier. Examine each tube at the end of  $24 \pm 2$  hours and if no gas has formed, again at the end of  $48 \pm 3$  hours. Record presence or absence of gas formation at each examination of the tubes.

More detailed records of the amount of gas formed, though desirable for the purpose of study, are not necessary for performing the standard tests prescribed.

Formation within  $24 \pm 2$  hours of gas in the inverted vial in the fermentation tube constitutes a *Positive Presumptive Test*.

If no gas is formed in  $24 \pm 2$  hours, the incubation shall be continued to  $48 \pm 3$  hours. If gas in any quantity is present at the end of the second but not the first 24 hour incubation period, the test is considered as doubtful and the presence of organisms of the coliform group should be confirmed by means of the procedure described in "C" or "D," which follow.

The *absence of gas formation* at the end of  $48 \pm 3$  hours' incubation constitutes a *negative test*. (An arbitrary limit of 48 hours' observation doubtless excludes from consideration occasional members of the coliform group which form gas very slowly, but for the purpose of a standard test the exclusion of these occasional slow gas-forming organisms is considered immaterial.)

### C. CONFIRMED TEST

The use of Endo or eosin methylene-blue plates, or of the liquid confirmatory brilliant-green lactose bile is permitted.

Crystal violet broth may be used as an alternate medium where its use has been shown to yield a maximum number of coliform organisms as indicated by a series of completed tests.\*

\* See *Standard Methods for the Examination of Water and Sewage*, Eighth edition, page 203, for the preparation of this medium.

It is desirable that all lactose broth tubes showing gas at the end of 24 hours and those showing gas only at the end of 48 hours' incubation be submitted to this test. In routine work, however, it is permissible to submit to the Confirmed Test all the lactose broth tubes showing gas, of those containing the two highest dilutions (dilutions containing the smallest portions) of sample *that have produced gas*.

Thus, if only one or two dilutions of sample have been planted in lactose broth tubes, all tubes showing gas shall be confirmed, but if three or more dilutions of samples have been planted, only the tubes showing gas from the two highest gas-producing dilutions of sample need be submitted to the Confirmed Test.

For example, if five tubes of each of three dilutions are planted, and if gas appears in all tubes, the five tubes of the highest dilution and the five tubes of the middle dilution should all be confirmed. Again, if gas appears in only one of the five tubes of the highest dilution, three of the middle dilution, and four of the lowest dilution, only the one tube of the highest and the three of the middle dilution showing gas need be confirmed.

In such cases all remaining lactose broth tubes showing gas that have not been submitted to the Confirmed Test shall be recorded as containing coliform organisms, even though all the Confirmed Tests made yield negative results.

Transfers from the lactose broth tubes to plates or to confirmatory liquid media should be made *as soon as gas appears*. In routine work,



however, it is permissible to make observations and transfers at  $24 \pm 2$  hours and  $48 \pm 3$  hours of incubation.

### 1. Procedure

1.1. Endo or eosin methylene-blue plates. Streak one or more plates from each of the selected tubes showing gas formation in lactose broth; it is essential that the plates be so streaked as to insure the presence of some discrete colonies, separated by at least 0.5 cm. from one another. (See D, 1, below.)

1.1.1. Incubate the plates at  $35^{\circ}$  C. to  $37^{\circ}$  C. for  $24 \pm 2$  hours.

1.1.2. Results, typical (*Escherichia coli*) or atypical.

If typical *Escherichia coli* colonies have developed on the plate within the incubation period of  $24 \pm 2$  hours, the result of the confirmed test may be considered *positive*.

If only atypical colonies have developed within  $24 \pm 2$  hours, the result cannot yet be considered definitely negative, since many coliform organisms fail to form typical colonies on Endo or eosin methylene-blue plates, or the colonies develop slowly. In such case, it is always necessary to complete the test as directed under "D," below.

1.2. Brilliant-green lactose bile broth. Transfer from the lactose broth tube showing gas to a fermentation tube containing brilliant-green lactose bile broth.

When making transfers from the lactose broth tube showing gas, the tube shall first be gently shaken, or mixed by rotating, and the transfer shall be made by means of a wire loop not less than 3 mm. in diameter.

1.2.1. Incubate the inoculated bril-

liant-green lactose bile broth tube for  $48 \pm 3$  hours at  $35^{\circ}$  C. to  $37^{\circ}$  C.

1.2.2. The formation and presence of gas in any amount in the inverted vial of the fermentation tube at any time within  $48 \pm 3$  hours constitutes a *Confirmed Test*.

1.2.3. If the brilliant-green lactose bile broth tube is decolorized before or at the end of the 48 hour incubation period, the Completed Test, as in "D" below, should immediately be performed. (It has been suggested that when such decolorization, probably due to *Cl. welchii*, takes place, a transfer should be made at once to broth containing 2 per cent dried bile and 1:25,000 brilliant-green or to formate ricinoleate broth. If gas appears within  $48 \pm 3$  hours at  $35^{\circ}$  C. to  $37^{\circ}$  C., the culture should be submitted to the Completed Test.)

### D. COMPLETED TEST

#### 1. Procedure

The Completed Test may be performed upon the lactose broth tubes showing gas, the colonies found upon plates used for the Confirmed Test (C, 1.1), or the brilliant-green lactose bile broth tubes, showing gas, used for the Confirmed Test (C, 1.2).

1.1. Lactose broth tubes. If the lactose broth tubes are used for the Completed Test, the choice of these tubes to be tested shall be that specified for the Confirmed Test in "C."

1.1.1. Streak one or more Endo or eosin methylene-blue plates from each lactose broth tube to be tested. Careful attention to the following details, when streaking plates, will result in a high proportion of successful isola-

tions if coliform organisms are present:

a. Employ a straight needle slightly curved at the tip. By bringing only the curved section of the needle in contact with the agar surface, the latter will not be scratched or torn.

b. Incline the lactose broth tube to avoid picking up any membrane or scum on the needle.

c. Insert the end of the needle into the liquid in the tube to a depth of approximately 1.0 mm.

d. Then streak the plate, covering completely the whole agar surface.

Incubate the plate (inverted, if without porous cover) at 35° C. to 37° C. for  $24 \pm 2$  hours.

1.2. Brilliant-green lactose bile broth tubes. If the brilliant-green lactose bile broth tubes used for the Confirmed Test are to be employed for the Completed Test, streak one or more Endo or eosin methylene-blue plates from each brilliant-green lactose bile broth tube showing gas, as soon as possible after appearance of gas, following closely the directions indicated in 1.1. Incubate the plates at 35° C. to 37° C. for  $24 \pm 2$  hours.

1.3. Identification. From each of the plates used for the Confirmed Test, or from those made from the lactose broth or brilliant-green lactose bile broth tubes (D, 1.1 or 1.2), fish one or more typical *Escherichia coli* colonies; or, if no such typical colonies are present, fish two or more colonies considered most likely to consist of organisms of the coliform group, transferring each to an agar slant and a lactose broth fermentation tube.

When transferring colonies, care should be taken to choose, if possible,

well isolated colonies separated by at least 0.5 cm. from other colonies, and barely to touch the surface of the colony with the needle in order to minimize the danger of transferring a mixed culture.

The secondary lactose broth fermentation tubes thus inoculated shall be incubated at 35° C. to 37° C. until gas formation is noted—the incubation not to exceed  $48 \pm 3$  hours.

The agar slants shall likewise be incubated at 35° C. to 37° C. for 24 to  $48 \pm 3$  hours, and Gram-stained preparations from those corresponding to the secondary lactose broth tubes that show gas shall be examined microscopically.

1.4. Results. The formation of gas in lactose broth and the demonstration of Gram-negative, non-spore-forming bacilli in the agar culture shall be considered a satisfactory Completed Test, demonstrating the presence of a member of the coliform group.

The absence of gas formation in lactose broth or failure to demonstrate Gram-negative non-spore-forming bacilli in a gas-forming culture constitutes a *negative test*.

When spore-forming lactose-fermenting organisms are found, the culture should be further studied to ascertain the possible presence of bacteria of the coliform group associated with the spore-bearing organisms. This may be done by transferring the culture to formate ricinoleate broth and incubating at 35° C. to 37° C. for  $48 \pm 3$  hours.

If no gas is produced, only spore-forming lactose fermenters may be considered to be present. If gas is

produced in the formate ricinoleate broth, the probable presence of coliform group organisms should be verified by inoculation from the formate ricinoleate to a tube of standard lactose broth and to an agar slant.

If, after  $48 \pm 3$  hours, gas is produced in the former and no spores in the latter, the test may be considered "completed" and the presence of coliform organisms demonstrated.

If spores are present, for practical purposes, organisms of the coliform group may be considered absent.

#### E. TECHNIC FOR THE GRAM STAIN

The "Completed Test" for coliform group organisms includes the determination of Gram stain characteristics of the organisms isolated (Part IV, Sec. 9, D, 1.3, page 197).

*The Manual of Methods for Pure Culture Study of Bacteria*, issued by the Society of American Bacteriologists (Geneva, N. Y.) includes a section on staining procedures (Leaflet IV, May, 1939). By permission, a portion of the section relating to the Gram stain is included herewith.

A word of caution is necessary regarding the interpretation of Gram stain results. Organisms are so generally recorded in the literature as either Gram-positive or Gram-negative, that this stain is often considered to give a clear-cut reaction as definite as a chemical test. Many organisms, however, are actually Gram-variable; and to determine their predominant tendency in this respect, repeated tests are needed.

There are a large variety of modifications of the Gram stain, many of which have been listed by Hucker

and Conn. The following Hucker modification is valuable for staining smears of pure cultures.

#### 1. Reagents

1.1. Ammonium oxalate crystal violet.

1.1.1. Solution A. Dissolve 2 g. of crystal violet, with 85 per cent dye content, in 20 ml. of 95 per cent ethyl alcohol.

1.1.2. Solution B. Dissolve 0.8 g. of ammonium oxalate in 80 ml. of distilled water.

1.1.3. Mix solutions A and B, ordinarily in equal parts. It is sometimes found, however, that this gives so concentrated a stain that Gram-negative organisms, such as the gonococcus, do not properly decolorize. To avoid this difficulty, solution A may be diluted as much as ten times, and 20 ml. of the diluted solution mixed with an equal quantity of solution B.

1.2. Lugol's Solution; Gram's Modification. Dissolve 1 g. of iodine crystals, and 2 g. of potassium iodide in 300 ml. of distilled water.

1.3. Counterstain. Make an alcoholic solution of safranin dye by dissolving 2.5 g. in 100 ml. of 95 per cent ethyl alcohol.

Add 10 ml. of the alcoholic solution of safranin to 100 ml. of distilled water.

#### 2. Procedure

Stain the smear for 1 minute with the crystal violet solution. Wash slide in water; immerse in iodine solution for 1 minute.

Wash stained slide in water; blot dry. Decolorize with 95 per cent



ethyl alcohol for 30 seconds; use gentle agitation.

Blot and cover with counterstain for 10 seconds. Then wash, dry, and examine as usual.

Cells which decolorize and accept the safranin stain are Gram-negative. Cells which do not decolorize, but retain the crystal violet stain, are Gram-positive.

#### F. SELECTION OF COLIFORM TESTS

The laboratory worker, when he elects to apply either the Presumptive, Confirmed or the Completed Test for the coliform group, shall be guided by the following basic considerations.

##### 1. *Presumptive Test*

The Presumptive Test may be applied to gas-forming portions of:

- any sample of sewage, sewage effluent (except chlorinated effluent), or water known to be heavily polluted, the fitness of which for use as drinking water is not under consideration.
- any routine sample of raw water in a purification plant, provided that records indicate the Presumptive Test to be not too inclusive for the production of data statistically comparable to that obtained from the finished water.

##### 2. *Confirmed Test*

The Confirmed Test may be applied to the gas-forming portions indicated in Part IV, Sec. 9, C, page 195:

- in the examination of any water to which the Presumptive Test is

known, from previous records, to be not applicable.

- in the routine examination of samples of drinking water, water in process of purification and finished waters.
- in the examination of chlorinated sewage effluents.

##### 3. *Completed Test*

The Completed Test shall be applied to the gas-forming portions indicated in Part IV, Sec. 9, C, page 195:

- in the examination of any water to which the applicability of the Confirmed Test is in reasonable doubt. Laboratories responsible for the quality of the raw or finished waters supplied to large communities shall employ the Completed Test, if not exclusively to these raw or finished waters, at least to such a proportion of samples as to establish beyond reasonable doubt the applicability to them of the Confirmed Test.

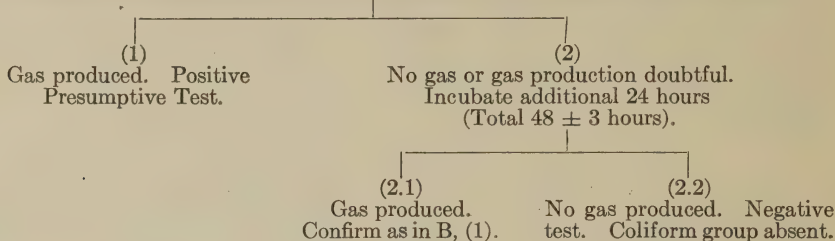
#### BIBLIOGRAPHY

- HUCKER, G. J., AND CONN, H. J. Methods of Gram staining. *New York Agr. Exp. Sta. Tech. Bul.* 93 (1923).
- NORTON, J. F., AND WEIGHT, J. J. Aerobic spore forming lactose fermenting organisms and their significance in water analysis. *Am. J. Public Health*, 14, 1019 (1924).
- HUCKER, G. J., AND CONN, H. J. Further studies on the methods of Gram staining. *New York Agr. Exp. Sta. Tech. Bul.* 128 (1927).
- BERGEY, BREED, MURRAY, AND HITCHENS. *Bergey's Manual of Determinative Bacteriology*, 5th Ed., 388 (1939).
- COWLES, P. B. A modified fermentation tube. *J. Bact.*, 38, 677 (1939).

# 10. Schematic Outline of Presumptive, Confirmed, and Completed Tests as Described in Section 9

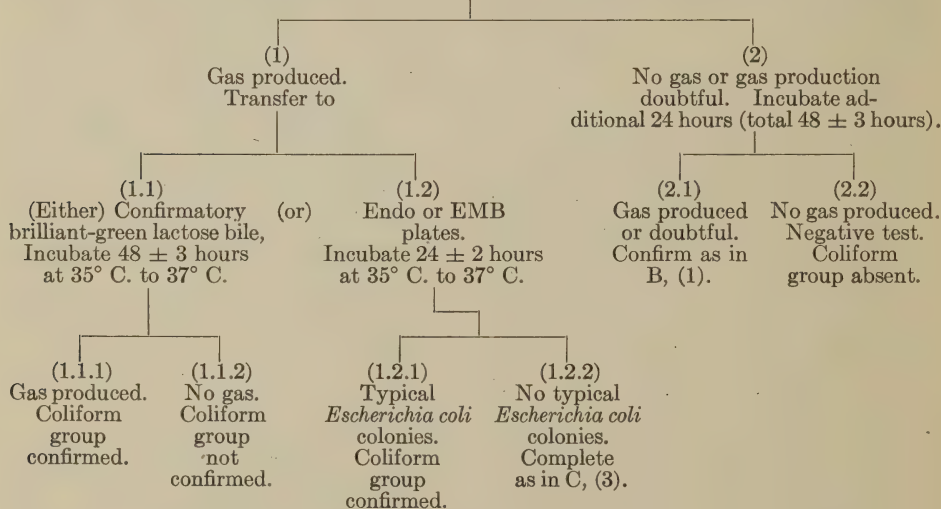
## A. PRESUMPTIVE TEST.

Inoculate lactose broth fermentation tubes and incubate  $24 \pm 2$  hours at  $35^{\circ}\text{C.}$  to  $37^{\circ}\text{C.}$



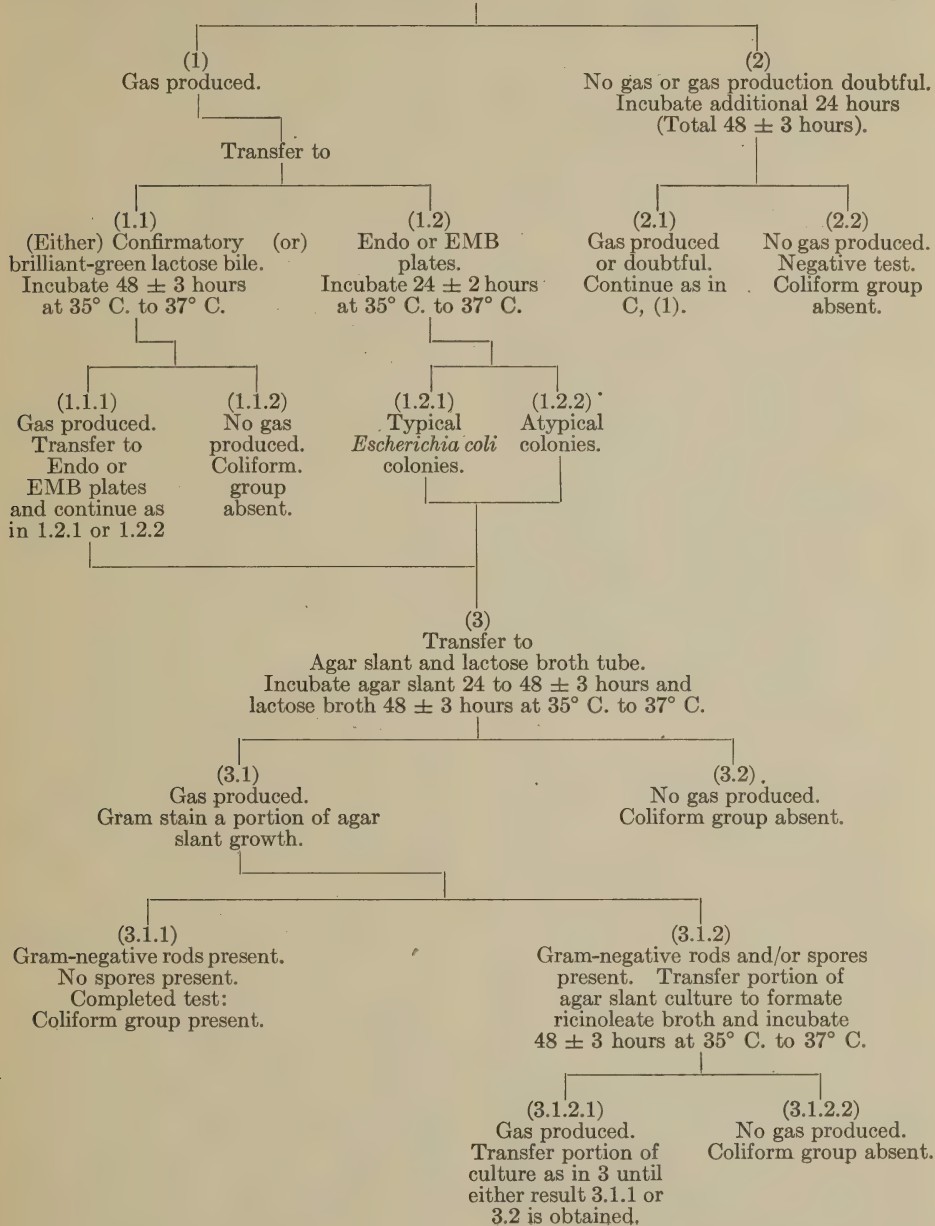
## B. CONFIRMED TEST.

Inoculate lactose broth fermentation tubes and incubate  $24 \pm 2$  hours at  $35^{\circ}\text{C.}$  to  $37^{\circ}\text{C.}$



## C. COMPLETED TEST.

Inoculate lactose broth fermentation tubes and incubate  $24 \pm 2$  hours at  $35^{\circ}\text{C}$ . to  $37^{\circ}\text{C}$ .





## 11. Estimation of Coliform Group Density

### A. BASIS AND GENERAL CONSIDERATIONS

Tests for the presence and density of organisms of the coliform group shall be based on the primary inoculation into lactose broth of one or more portions of one or more decimal dilutions of the sample.

The number of portions planted and the range of the dilutions made will depend on the presumed character of the water under examination, as well as on the records or absence of records of previous samples. Every fermentation tube shall contain such an amount of medium and such an initial concentration of ingredients that, after the addition of the sample, the concentration of the ingredients in the mixture shall be that specified in the formula given for the preparation of the medium (Part IV, Sec. 3, N, 2, page 189).

### B. WATER OF PRESUMED DRINKING WATER QUALITY

When examining water of presumed drinking water quality, inoculate a series of lactose broth fermentation tubes with appropriate graduated quantities of the water to be tested.

The amounts of sample selected for inoculating should be such that the largest portions will result in gas production in all or the majority of broth tubes into which they are inoculated, and such that the smallest portions will result in no gas production in all or the majority of broth tubes into which these smallest portions are inoculated. Because the numerical value of the bacterial content is

largely determined by the analytical result of that dilution or those dilutions of the sample intermediate between the two above mentioned, an ample number of tube plantings should be made of the intermediate or critical portions.

The number of such critical portions to be inoculated will be governed by the desired accuracy of the result (and in general should be not less than 5), whereas the numbers of the largest and smallest portions to be inoculated will be governed by the probable accuracy of the estimate of the bacterial content at the time the series of dilutions is decided upon.

Inasmuch as definitive indications of the occurrence of occasional higher densities of the coliform group are desirable and are facilitated by multiple plantings of the highest dilution, more than one and up to five such portions may be inoculated. Practical considerations limit the size of the largest portions to 100 ml.; and even when 100 ml. portions are inoculated, it is possible that gas production will not result in the majority of the broth tubes.

### C. WATER SUBJECT TO USPHS DRINKING WATER STANDARDS

When examining water for evidence of conformance to the U. S. Public Health Service Drinking Water Standards, inoculate a series of 5 *lactose broth* fermentation tubes, each with a 10 ml. or a 100 ml. portion of the sample. The confirmed test or the completed test must be employed for examination of these samples. The results are recorded in terms of the number of positive por-

tions of each sample examined. For purpose of record the results should be stated also in terms of the most probable number of coliform group organisms present.

#### D. WATER OF OTHER THAN DRINKING WATER QUALITY

When examining water of other than drinking water quality, inoculate a series of lactose broth fermentation tubes with appropriate graduated quantities of the water to be tested. The principles stated in Sec. 11, B, page 202, relative to the selection of portions to be examined, apply here. The selection of the size of the portions to be tested will depend upon the probable coliform group density in the sample and will be governed by the technician's experience of the character of the water under examination.

#### E. COMPUTING AND RECORDING THE MOST PROBABLE NUMBER

The number of positive findings of coliform group organisms (either presumptive or confirmed) resulting from multiple portion decimal dilution plantings made according to Sec. 11, B, C, or D above, shall be computed and recorded in terms of the "Most Probable Number" (M.P.N.). The most probable number results for a variety of planting series and positive results are shown in Table 17. Where the terms low, mid, and high are used at the head of a column, they refer to the low, mid, or high dilutions and indicate respectively the greatest, middle and least portion of the original sample.

The quantities indicated at the

heads of the columns relate more specifically to finished waters. The figures may be used in computing the M.P.N. in larger or smaller portion plantings in the following manner: if instead of 10, 1.0 and 0.1 ml. portions, a combination of 100, 10 and 1 ml. portions is used, the M.P.N. may be recorded as 0.1 times the figure in the table.

If, on the other hand, a combination of corresponding portions of 1.0, 0.1 and 0.01 ml. is planted, record 10 times the figure in the table; if a combination of 0.1, 0.01 and 0.001 ml. portions is planted, record 100 times the figure in the table.

When more than three dilutions are employed, the results from only three are significant. To choose these, taking the system of 5 tubes at each dilution for example, the highest 5/5 below which no smaller result occurs should be taken, and also the two following. (The number in the numerator represents positive tubes, that in the denominator, the total tubes planted.) In the examples given below the significant dilution results are shown in bold-face type:

	1 ml.	0.1 ml.	0.01 ml.	0.001 ml.
(a)	<b>5/5</b>	<b>5/5</b>	<b>2/5</b>	0/5
(b)	<b>5/5</b>	<b>4/5</b>	<b>2/5</b>	0/5
(c)	<b>0/5</b>	<b>1/5</b>	<b>0/5</b>	0/5

In the example (c) above, the first three dilutions should be taken, so as to throw the positive result in the middle dilution.

When a case such as the following (d) arises, where a positive occurs in a dilution higher than the three chosen according to the rule, it should be included in the result of the high-

est chosen dilution, making the result read as in example (e) :

	1 ml.	0.1 ml.	0.01 ml.	0.001 ml.
(d)	5/5	3/5	1/5	1/5
(e)	5/5	3/5	2/5	0/5

The most desirable procedure for obtaining a single numerical value for

a series of analytical results is to express the results of each analysis in terms of its M.P.N. value and strike an arithmetic average of these values. (It is mathematically incorrect to summarize the number of positive tubes in the various dilutions for a

TABLE 17a.—MOST PROBABLE NUMBERS—COLIFORM GROUP TESTS  
Most probable numbers per 100 ml. of sample, planting various portions in not more than 3 dilutions

Number of positive tubes in dilutions			Combinations of portions planted in ml.			Number of positive tubes in dilutions			Combinations of portions planted in ml.				
Low	Mid	High	2-10 2-1 2-0.1	1-10 5-1 1-0.1	1-50 5-10 1-1	Low	Mid	High	5-10	5-10 1-1	5-10 1-1 1-0.1	5-100 1-50 1-10	5-100 5-10
0	0	1	4.5	6.7	1.0	0	0	1			2.0	.18	
0	0	2	9.0			0	1	0		2.0	2.0	.19	.18
0	1	0	4.6	6.8	1.0	0	2	0					.37
0	1	1	9.2	14.	2.1	0	3	0					.56
0	1	2	14.			0	4	0					.75
0	2	0	9.4	14.	2.2	0	1	1			4.0	.38	
0	2	1	14.	21.	3.3	1	0	0	2.2	2.2	2.2	.20	.20
0	2	2	19.			1	0	1			4.4	.40	
0	3	0		22.	3.5	1	1	0		4.4	4.4	.42	.41
0	3	1		30.	4.7	1	2	0					.62
0	4	0		31.	5.0	1	3	0					.84
0	4	1		39.	6.4	1	4	0					1.1
0	5	0		40.	6.8	1	1	1			6.7	.63	
0	5	1		49.	8.3	2	0	0	5.1	5.0	5.0	.44	.45
1	0	0	6.0	11.	1.4	2	0	1			7.5	.68	
1	0	1	12.	24.	2.9	2	1	0		7.6	7.6	.71	.69
1	0	2	19.			2	2	0					.94
1	1	0	13.	26.	3.1	2	3	0					1.2
1	1	1	20.	45.	4.9	2	4	0					1.5
1	1	2	28.			2	1	1			10.	.97	
1	2	0	21.	51.	5.5	3	0	0	9.2	8.9	8.8	.77	.79
1	2	1	29.	76.	7.9	3	0	1			12.	1.1	
1	2	2	37.			3	1	0		12.	12.	1.1	1.1
1	3	0		89.	9.0	3	2	0					1.4
1	3	1		120.	12.	3	3	0					1.8
1	4	0		150.	15.	3	4	0					2.1
1	4	1		210.	21.	3	1	1			16.	1.5	
1	5	0		390.	39.	4	0	0	16.0	15.	15.	1.3	1.3
2	0	0	23.			4	0	1			20.	1.7	
2	0	1	50.			4	1	0		21.	21.	1.8	1.7
2	0	2	95.			4	2	0					2.2
2	1	0	62.			4	3	0					2.8
2	1	1	130.			4	4	0					3.5
2	1	2	210.			4	1	1			27.	2.4	
2	2	0	240.			5	0	0		39.	38.	2.2	2.4
2	2	1	700.			5	0	1			96.	3.1	
						5	1	0			240.	4.7	3.5
						5	2	0					5.4
						5	3	0					9.2
						5	4	0					16.0

From "Most probable numbers for evaluation of Coli-Aerogenes Tests by Fermentation Tube Method," *Reprint No. 1621, Pub. Health Repts.*, 49, 393-405 (1934, revised 1940), and *Pub. Health Repts.*, 40, 693-721 (1925) *Reprint No. 1029, p. 14.*





coliform organisms may be required. In such cases, the "Indicated Number" (I.N.) may be used.

The number obtained by taking the reciprocal of the smallest positive dilution in a decimal series gives an indication of the coliform group density in the sample. (If multiple portions are planted in each dilution, the reciprocals are averaged.) The number of organisms so obtained shall be recorded as the "indicated number" (I.N.).

In assembling such results, if an anomalous finding occurs, i.e., a negative result in a portion larger than the smallest portion giving a positive result, these findings are reversed. For example, if the results are 10 ml., positive; 1 ml., negative; and 0.1 ml., positive; the observations are unreasonable, and the results should be recorded as 10 ml., positive; 1 ml., positive; and 0.1 ml., negative.

The following table illustrates the method of recording results on (1) five separate samples using single portions in each dilution and (2) a single sample using five portions in each of four dilutions.

Results of Tests in Amounts Designated				Indicated Number of Organisms of the Coliform Group.	
1 ml.	0.1 ml.	0.01 ml.	0.001 ml.	per ml.	per 100 ml.
+	-	-	-	1	100
+	+	-	-	10	1,000
+	+	+	-	100	10,000
+	+	+	+	1000	100,000
+	+	-	+	100	10,000

Such widely varying results as above should not be averaged, but from more concordant results, the indicated numbers per ml. or per 100 ml. may be totaled and numerically averaged (and adjusted to significant figures).

In order that results as reported may be checked and carefully evaluated, it is necessary that the report should show not only the average number of organisms per ml., but also the number of samples examined; and, for each dilution, the total number of tests made, and the number (or per cent) positive.

#### G. PRECISION OF THE FERMENTATION TUBE TEST

It is desirable to keep in mind the fact that, unless a large number of portions of sample are examined, the precision of the fermentation tube test is rather low. For example, even when the sample contains one coliform organism per ml., about 37 per cent of 1 ml. tubes may be expected to yield negative results, because of irregular distribution of the bacteria in the sample. When 5 tubes, with 1 ml. of sample in each, are employed under these conditions, a completely negative result may be expected less than 1 per cent of the time.

Even when 5 fermentation tubes are employed, however, the precision of the result obtained is not of a high order. Consequently, great caution must be exercised when interpreting, in terms of sanitary significance, the coliform results obtained from the use of a few tubes with each dilution of sample.

#### BIBLIOGRAPHY

- MCCRADY, MAC H. The numerical interpretation of fermentation tube results. *J. Infect. Dis.*, 17, 183 (1915).  
GREENWOOD, M., AND YULE, G. U. On the statistical interpretation of some bacteriological methods employed in water analysis. *J. Hyg.*, 16, 36 (1917).

- WOLMAN, A., AND WEAVER, H. L. A modification of the McCrady method of the numerical interpretation of fermentation tube results. *J. Infect. Dis.*, 21, 287 (1917).
- MCCRADY, MAC H. Tables for rapid interpretation of fermentation-tube results. *Can. Pub. Health J.*, 9, 201 (1918).
- REED, L. J. B. coli densities as determined from various types of samples. *Pub. Health Repts.*, 40, 704 (1925). Reprint No. 1029.
- HOSKINS, J. K. The most probable number of B. coli in water analysis. *J. Amer. W. W. Assn.*, 25, 867 (1933).
- HOSKINS, J. K. Most probable numbers for evaluation of coli-aerogenes tests by fermentation tube method. *Pub. Health Repts.*, 49, 393 (1934). Reprint No. 1621.
- HALVORSON, H. O., AND ZIEGLER, N. R. Application of statistics to problems in bacteriology. *J. Bact.*, 25, 101 (1933); 26, 331 (1933); 26, 559 (1933); 29, 609 (1935).
- HOSKINS, J. K., AND BUTTERFIELD, C. T. Determining the bacteriological quality of drinking water. *J. Amer. W. W. Assn.*, 27, 1101 (1935).
- SWAROOP, S. Numerical estimation of B. coli by dilution method. *Indian J. Med. Res.*, 26, 353 (1938).
- DALLA VALLE, J. M. Notes on the "Most probable number" index as used in bacteriology. *Pub. Health Repts.*, 56, 229 (1941).
- THOMAS, HAROLD A., JR. Bacterial densities from fermentation tube tests. *J. Amer. W. W. Assn.*, 34, 572 (1942).
- PUBLIC HEALTH SERVICE DRINKING WATER STANDARDS. *Pub. Health Repts.*, 61, 371 (1946).

## 12. Interpretation of Coliform Group Results

It is not within the province of this text to suggest the proper interpretation of results obtained by the use of the methods herein specified as standard. Reference may be made to the U. S. Public Health Service Drinking Water Standards for drinking water on interstate carriers for a discussion of permissible density of organisms of the coliform group.

The definition of the coliform group as given includes organisms of both the so-called "fecal" and "non-fecal" types. At the present time, any attempt to evaluate a drinking water on the basis of a distinction between these two types is regarded as unwarranted. In order to stimulate further work along this line, however, the media and methods in common use for the differentiation of organisms of the coliform group are included in Appendix I, Secs. 13 and 14, pages 227-233. The inclusion of these methods should not be construed as detracting from the value of the group tests as above described for the routine examination of water supplies.

## BIBLIOGRAPHY

- KEHR, R. W., AND BUTTERFIELD, C. T. Notes on the relation between coliforms and enteric pathogens. *Pub. Health Repts.*, 58, 589 (1943).
- PUBLIC HEALTH SERVICE DRINKING WATER STANDARDS. *Pub. Health Repts.*, 61, 371 (1946).

## 13. Bacteriological Control of Swimming Pools and Bathing Places

### A. PREPARATION OF THE SAMPLE BOTTLE

All samples from swimming pool water containing chlorine shall be collected in bottles containing sodium thiosulfate. (Part IV, Sec. 4, A, page 190.) The purpose of the sodium thiosulfate is to destroy the chlorine present in a treated water supply at the moment the sample is collected. This thiosulfate prevents a continuation of the killing action of the chlorine on the bacteria while the sample



is being transported to the laboratory. The bacteriological examination then gives the true sanitary quality of the water at the time the sample was collected.

#### B. COLLECTION OF SAMPLES

The samples should be collected by plunging the open bottle beneath the surface, sweeping the bottle forward until filled. *Do not rinse the bottle in the pool, otherwise the sodium thiosulfate will be removed.*

Samples should be collected only when the pool is in use and preferably during periods of the heaviest load for the day.

The residual chlorine test shall be made at the pool side at the time the sample is collected.

All samples shall be tested for residual chlorine at the laboratory at the time of bacteriological analysis as a check against the removal of sodium thiosulfate at the time of collecting the sample.

#### C. DETERMINATION OF THE BACTERIAL COUNT

The bacterial count at 35°-37° C. shall be made in the same manner as for other types of water. (Part IV, Sec. 6, page 191.)

#### D. DETERMINATION OF THE COLIFORM GROUP

The method of procedure and media shall be those recommended for other types of water. Part IV, Sec. 9, page 193.

## APPENDIX I

### NON-STANDARD METHODS

#### Foreword

It has been the policy in the "standard" sections of "Standard Methods for the Examination of Water and Sewage," to include no methods which have not reached a state of reasonably general acceptance in the laboratory field. It is not presumed that the "standard" section in any edition represents material on which the last word has been said, but it is presumed that the methods therein included have been studied with sufficient diligence and subjected to a sufficient degree of critical examination to indicate their availability as routine and correct procedures.

The policy adopted in the Seventh Edition of this text, namely, that of adding an appendix of provisional or non-standard procedures, is continued in this Edition. In this category are included methods which, in the opinion of the Editorial Committee, are approaching, but have not yet reached a state of general acceptance that would make it possible to include them in the "standard" text.

Laboratory workers must distinctly understand the difference between the "standard" and the provisional methods. The provisional methods are only to be used to supplement the "standard" methods, they are not a substitute for them.

It is a matter of regret that some of the determinations in this category

have not been widely enough studied to permit certain defects in them to be adjusted and to permit their inclusion in the standard text. It is hoped that their inclusion under this heading will stimulate laboratory workers to use them and to improve them.

It has not been the intention to outline these provisional methods with the degree of exactness that would permit the laboratory worker to use this text as a guide to actual performance. References have been given which will not only outline the method in the originator's own words, but will also acquaint the workers with the character of the study which has already been given to the subject.

In addition, a section has been included in order to give American workers some indication of English practice in bacteriological examinations.

It is recognized that procedures may have been omitted from this category that have an especial appeal to some workers. It is regretted that limitations of space do not permit the inclusion of all pertinent material.

#### 1. Chromium in Water

##### 1. *Reagents*

1.1. S-diphenyl carbazide. Dissolve 0.4 g. of S-diphenyl carbazide in 30 ml. of glacial acetic acid and dilute to 400 ml. with distilled water.

## 2. Procedure

Acidify a measured volume of from 1 to 10 liters of water with  $\text{HNO}_3$  and evaporate to dryness. Dissolve the residue in  $\text{HNO}_3$ , neutralize with  $\text{KOH}$  soln., adding a slight excess. Then add 5 ml. of concd.  $\text{H}_2\text{O}_2$  and heat.

Since the metal hydroxides precipitated by this treatment retain some of the chromium mechanically, it is necessary to filter and dissolve the residue in dil.  $\text{HNO}_3$  and again neutralize and oxidize.

Combine the filtrates, neutralize with acetic acid, adding a slight excess. On adding lead acetate the chromate is precipitated quantitatively.

Dissolve the well washed lead precipitate in  $\text{HCl}$  and make up to a given volume in a volumetric flask.

The chromium content may be estimated colorimetrically by comparison with standard potassium chromate solution, using the S-diphenyl carbazide reagent.

Add 1 ml. of the 0.1 per cent solution of reagent to a measured amount of the chromate solution. Allow to stand for 15 minutes and dilute with distilled water to 50 ml. for final reading.

If 50 ml. Nessler tubes are used for comparison the amounts that may be read with greatest accuracy have been found to be from 0.003 mg. to 0.01 mg. in a 50 ml. tube with 0.007 mg. representing an ideal amount.

## BIBLIOGRAPHY

AKATSUKA, K., AND FAIRHALL, L. T. J.  
*Ind. Hyg.*, 16, 1 (1934).

## 2. Selenium in Water

Small amounts of selenium are best estimated colorimetrically.

### 1. Reagents

1.1. Gum arabic solution, 5 per cent.

1.2. Hydroxylamine hydrochloride.

1.3. Sulfur dioxide.

1.4. Hydrobromic acid, concd.

### 2. Procedure

Measure from 1 to 10 liters of water, add sufficient sodium peroxide to make the liquid definitely alkaline. Evaporate to dryness. Take up with 100 ml. of concentrated hydrobromic acid and 1 to 5 ml. of bromine, depending upon the quantity of organic matter present, transfer to a distilling flask and collect 50 to 75 ml. of the distillate.

Add 1 ml. of 5 per cent gum arabic solution and precipitate the selenium in the distillate by means of sulfur dioxide and hydroxylamine hydrochloride.

Prepare comparison solutions containing known quantities of selenium in exactly the same manner and allow them to stand overnight. Shake the standard and test solutions and compare the depth of color in Nessler tubes.

This comparison is best carried out in sunlight. It is difficult to match solutions containing more than 0.5 mg. of selenium in 25 ml. The color comparison is most satisfactory when 0.01 to 0.10 mg. is present.



## BIBLIOGRAPHY

- ROBINSON, W. O., DUDLEY, H. C., WILLIAMS, K. T., AND BYERS, H. G. *Ind. Eng. Chem., Anal. Ed.*, 6, 274 (1934).  
DUDLEY, H. C., AND BYERS, H. G. *Ind. Eng. Chem., Anal. Ed.*, 7, 3 (1935).

## 3. pH Buffers and Indicators

There are so many uses for standard buffer mixtures and the various indicators that it appears desirable to supplement the information given in Part I, Sec. 11, B, page 30, with some data \* on these subjects.

## A. BUFFER STANDARDS

The various mixtures are made up from the following stock solutions: 0.2 M potassium chloride ( $\text{KCl}$ ), 0.2 M acid potassium phosphate ( $\text{KH}_2\text{PO}_4$ ), 0.2 M acid potassium phthalate ( $\text{KHC}_8\text{H}_4\text{O}_4$ ), 0.2 M boric acid with 0.2 M potassium chloride ( $\text{H}_3\text{BO}_3$ ,  $\text{KCl}$ ), 0.2 M sodium hydroxide ( $\text{NaOH}$ ), and 0.2 M hydrochloric acid ( $\text{HCl}$ ).

1. 0.2 M potassium chloride solution. The salt should be recrystallized three or four times and dried in an oven at about  $120^\circ\text{C}$ . for two days. The 0.2 M solution contains 14.912 g. in 1 liter.

2. 0.2 M acid potassium phthalate solution. Acid potassium phthalate of the grade of Bureau of Standards, standard sample no. 84 shall be used. Dry the salt at  $110$ – $115^\circ\text{C}$ . to constant weight. A 0.2 M solution contains 40.836 g. of the salt in 1 liter of the solution.

3. 0.2 M acid potassium phosphate solution. A high grade commercial

sample of the salt is recrystallized at least three times from distilled water and dried to constant weight at  $110$ – $115^\circ\text{C}$ . A 0.2 M solution should contain 27.232 g. in 1 liter. The solution should be distinctly red with methyl red and distinctly blue with brom phenol blue.

4. 0.2 M boric acid, 0.2 M potassium chloride. Boric acid should be recrystallized several times from distilled water. It should be air dried in thin layers between filter paper and the constancy of weight established by drying small samples in thin layers in a desiccator over  $\text{CaCl}_2$ . One liter of the solution should contain 12.4048 g.  $\text{H}_3\text{BO}_3$  and 14.912 g.  $\text{KCl}$ .

5. 0.2 M sodium hydroxide solution. Dissolve 100 g.  $\text{NaOH}$  in 100 ml. distilled water in a Pyrex glass Erlenmeyer flask. Cover the mouth of the flask with tin foil and allow the solution to stand overnight until the carbonate has settled. Then prepare a filter as follows.

Cut a "hardened" filter paper to fit a Buchner funnel. Treat it with warm, strong (1:1)  $\text{NaOH}$  solution. After a few minutes decant the sodium hydroxide and wash the paper first with absolute alcohol, then with dilute alcohol, and finally with large quantities of distilled water.

Place the paper on the Buchner funnel and apply gentle suction until the greater part of the water has evaporated; but do not dry so that the paper curls. Now pour the concentrated alkali upon the middle of the paper, spread it with a glass rod, making sure that the paper, under gentle suction, adheres well to the

\* The text and tables are paraphrased from the 3rd edition of *The Determination of Hydrogen Ions* by W. M. Clark, by permission of Williams & Wilkins Co. (Publishers).

TABLE 18.—COMPOSITION OF MIXTURES GIVING VARIOUS pH VALUES  
 From Clark (1928), p. 200-1

In all cases add boiled and cooled redistilled water to make 200 ml.					
pH	1.2	0.2M KCl	50 ml.	0.2M HCl	64.5 ml.
"	1.4	"	50 ml.	"	41.5 ml.
"	1.6	"	50 ml.	"	26.3 ml.
"	1.8	"	50 ml.	"	16.6 ml.
"	2.0	"	50 ml.	"	10.6 ml.
"	2.2	"	50 ml.	"	6.7 ml.
pH	2.2	0.2M KH Phthalate	50 ml.	0.2M HCl	46.70 ml.
"	2.4	"	50 ml.	"	39.60 ml.
"	2.6	"	50 ml.	"	32.95 ml.
"	2.8	"	50 ml.	"	26.42 ml.
"	3.0	"	50 ml.	"	20.32 ml.
"	3.2	"	50 ml.	"	14.70 ml.
"	3.4	"	50 ml.	"	9.90 ml.
"	3.6	"	50 ml.	"	5.97 ml.
"	3.8	"	50 ml.	"	2.63 ml.
pH	4.0	0.2M KH Phthalate	50 ml.	0.2M NaOH	0.40 ml.
"	4.2	"	50 ml.	"	3.70 ml.
"	4.4	"	50 ml.	"	7.50 ml.
"	4.6	"	50 ml.	"	12.15 ml.
"	4.8	"	50 ml.	"	17.70 ml.
"	5.0	"	50 ml.	"	23.85 ml.
"	5.2	"	50 ml.	"	29.95 ml.
"	5.4	"	50 ml.	"	35.45 ml.
"	5.6	"	50 ml.	"	39.85 ml.
"	5.8	"	50 ml.	"	43.00 ml.
"	6.0	"	50 ml.	"	45.54 ml.
"	6.2	"	50 ml.	"	47.00 ml.
pH	5.8	0.2M KH <sub>2</sub> PO <sub>4</sub>	50 ml.	0.2M NaOH	3.72 ml.
"	6.0	"	50 ml.	"	5.70 ml.
"	6.2	"	50 ml.	"	8.60 ml.
"	6.4	"	50 ml.	"	12.60 ml.
"	6.6	"	50 ml.	"	17.80 ml.
"	6.8	"	50 ml.	"	23.65 ml.
"	7.0	"	50 ml.	"	29.63 ml.
"	7.2	"	50 ml.	"	35.00 ml.
"	7.4	"	50 ml.	"	39.50 ml.
"	7.6	"	50 ml.	"	42.80 ml.
"	7.8	"	50 ml.	"	45.20 ml.
"	8.0	"	50 ml.	"	46.80 ml.
pH	7.8	0.2M H <sub>3</sub> BO <sub>3</sub> , M/5KCl	50 ml.	0.2M NaOH	2.61 ml.
"	8.0	"	50 ml.	"	3.97 ml.
"	8.2	"	50 ml.	"	5.90 ml.
"	8.4	"	50 ml.	"	8.50 ml.
"	8.6	"	50 ml.	"	12.00 ml.
"	8.8	"	50 ml.	"	16.30 ml.
"	9.0	"	50 ml.	"	21.30 ml.
"	9.2	"	50 ml.	"	26.70 ml.
"	9.4	"	50 ml.	"	32.00 ml.
"	9.6	"	50 ml.	"	36.85 ml.
"	9.8	"	50 ml.	"	40.80 ml.
"	10.0	"	50 ml.	"	43.90 ml.

funnel, and draw the solution through with suction.

The clear filtrate is now diluted quickly, after rough calculation, to a

solution somewhat more concentrated than 1 N. Withdraw 10 ml. of this dilution and standardize roughly with an acid solution of known strength,

or with a sample of acid potassium phthalate. From this approximate standardization calculate the amount required to furnish a 0.2 M solution.

Make the required dilution with the least possible exposure, and pour the solution into a paraffined bottle to which a calibrated 50 ml. burette and soda-lime guard tubes have been attached. The solution should now be most carefully standardized.

One of the simplest methods of doing this, and one which should always be used in this instance, is the use of acid potassium phthalate. Weigh out carefully on a chemical balance with standardized weights several portions of the salt of about 1.6 g. each.

Dissolve in about 20 ml. distilled water and add 4 drops phenolphthalein. Pass a stream of  $\text{CO}_2$ -free air through the solution and titrate with the alkali till a faint but distinct pink is developed. It is preferable to use a factor with the solution rather than

attempt adjustment to an exact 0.2 M solution.

6. 0.2 M hydrochloric acid solution. Dilute a high grade hydrochloric acid solution to about 20 per cent and distill. Dilute the distillate to approximately 0.2 M and standardize with the sodium hydroxide solution previously described. If convenient, it is well to standardize this solution carefully by the silver chloride method and check with the standardized alkali.

The only solution which it is absolutely necessary to protect from the  $\text{CO}_2$  of the atmosphere is the sodium hydroxide solution. Therefore all but this solution may be stored in ordinary bottles of resistant glass. The salt solutions, if adjusted to exactly 0.2 M may be measured from clean calibrated pipettes.

## B. INDICATORS

The characteristics of various indicators suitable for the range from pH 1.2 to pH 9.8, together with data con-

TABLE 19.—INDICATORS—pH RANGES FROM 1.2 TO 9.8

	A	B	C	Range pH	Color change	
					Acid	Alkaline
Meta cresol purple.....	382	26.2	1.51	1.2-2.8	red	yellow
Thymol blue.....	466	21.5	1.5	1.2-2.8	red	yellow
Brom phenol blue.....	669	14.9	3.98	3.0-4.6	yellow	blue
Brom cresol green.....	698	14.3	4.67	3.8-5.4	yellow	blue
Chlor phenol red.....	423	23.6	5.98	4.8-6.4	yellow	red
Brom phenol red.....	512	19.5	6.16	5.2-6.8	yellow	red
Brom cresol purple.....	540	18.5	6.3	5.2-6.8	yellow	purple
Brom thymol blue.....	624	16	7	6.0-7.6	yellow	blue
Phenol red.....	354	28.2	7.9	6.8-8.4	yellow	red
Cresol red.....	382	26.2	8.3	7.2-8.8	yellow	red
Meta cresol purple.....	382	26.2	8.32	7.4-9.0	yellow	purple
Thymol blue.....	466	21.5	8.9	8.0-9.6	yellow	blue
Ortho cresol phthalein.....	346		9.4	8.2-9.8	colorless	red

A. Molecular weight.

B. ml. of N/100 NaOH required per 0.1 g. indicator to form mono-sodium salt. Dilute to 250 ml. for 0.04 per cent reagent.

C. Approximate invert logarithm of apparent dissociation constant, i.e., pH about which the color changes of the indicator are sharpest.



cerning the method of preparing their mono-sodium salts for routine purposes are shown in Table 19.

#### 4. Lead

This method is based on the principle that when an ammoniacal cyanide solution of diphenylthiocarbazone is added to a solution containing lead, a red precipitate is formed which is soluble in chloroform or carbon tetrachloride. The color of this organic lead compound in chloroform varies with the amount present from a pale blue through a series of purples and reds to a deep cherry. Wichmann, Clifford and Vorhes have found the method suitable for the determination of lead on sprayed fruits and vegetables, and Ross and Lucas find it very satisfactory for analysis of biological materials. While the principle is the same in water work, several modifications and changes, in order to make it suitable for water analysis, seem to warrant detailed consideration of the procedure.

##### 1. Reagents

The reagents must be chemically pure and free from minute traces of lead.

1.1. Dithizone solution. Dissolve 50 mg. of diphenylthiocarbazone in 1 liter of U. S. P. chloroform. Keep tightly stoppered. The diphenylthiocarbazone can be obtained of sufficient purity from Eastman Kodak Company.

1.2. Ammonia reagent. Dissolve 10 g. of KCN and 10 g. of citric acid in 500 ml. of 28 per cent  $\text{NH}_4\text{OH}$  and dilute to 1 liter with distilled water.

This solution should be stored in a tight Pyrex glass container.

1.3. Standard lead solution. Dissolve 15.9855 g. of pure dry  $\text{Pb}(\text{NO}_3)_2$  crystals in one liter of distilled water which has been acidified with 1 ml. of concd.  $\text{HNO}_3$ . This is equivalent to 10 mg. of lead per ml. From this solution a standard solution may be made containing 0.01 mg. of lead per ml. Lead tends to precipitate in very dilute solutions. This weaker solution must be prepared each time determinations are made.

##### 2. Standards

Standards are prepared by using the standard lead solution (.01 mg. of lead per ml.). Ten ml. of dithizone solution and 2.5 to 3 ml. of ammonia reagent are added to a 50 ml. Nessler tube. This is diluted to the mark with water containing a fairly high salt concentration, in place of distilled water. A sodium chloride solution of approximately 200 ppm. has been found to be very satisfactory, or treated water known to be free from lead may be used.

Better results are obtained in matching standard and sample tubes when the salt concentration approaches that of ordinary waters, though within a range from 100 to 200 ppm. of a non-interfering salt ( $\text{NaCl}$ ,  $\text{CaCl}_2$ ,  $\text{CaSO}_4$ , etc.) the concentration makes little difference.

All tubes, both for standards and samples, must be corked tightly and shaken vigorously. Suggested values for the standard tubes are .000, .005, .010, .015, .025, .030, .040, .045 and .050 mg. of lead. The amounts of

lead may of course be varied to meet the necessary conditions.

Comparisons are made with standards by looking transversely through the chloroform layer. With a diffused light source (frosted glass) and standards of strength indicated above, it is not necessary to make up a duplicate tube by substituting pure chloroform for the dithizone solution with the sample.

While the dissolved salts in a water sample do change the hue of the dithizone slightly in some cases when there is no lead, the difference is slight. The color always remains green, although it may be lighter or darker than the zero standard. Where lead is present, even in very small amounts, no major differences in the red colors produced by lead are produced by these salts. This condition is not serious, since it does not affect the accuracy of the determination. It cannot be remedied by using a duplicate tube with chloroform instead of dithizone (placed behind the standard) as is sometimes done with a comparator block.

An ordinary Nessler tube rack with one side paneled serves for holding and comparing the sample tube with standards. This panel is fitted with a 3 x 4 inch piece of frosted glass which allows the light to pass through the chloroform layer. The entire rack and panel should be blackened with flat paint or acid proof wood stain. Such an arrangement proves very useful in distinguishing between very small color differences. If too great an accuracy is not desired, this apparatus, including the frosted glass, may easily be dispensed with. New lead

standards should be prepared each day, since the red color tends to fade.

### 3. Procedure

250 ml. of the sample are evaporated in a 500 ml. Erlenmeyer flask to 20 or 30 ml. over an open flame. The addition of 0.5 ml. of concd. HCl previous to this evaporation facilitates the operation and tends to prevent bumping. Best results are obtained if the samples are not allowed to go to complete dryness, since the solids may hardly be expected to redissolve in such a small volume of liquid as must necessarily be used in this test. After the concentrate cools, 10 ml. of ammonia reagent and 10 ml. of dithizone solution are added directly to the Erlenmeyer flask used for the evaporation. The flask is shaken vigorously for about 30 seconds and then its entire contents are transferred to a 50 ml. Nessler tube. This is diluted to the mark with distilled water, corked tightly and again agitated.

The chloroform solution of dithizone settles out rapidly, carrying dissolved lead present with it.\* It has been found convenient to run about

\* The adverse effect of varying pH or varying salt concentrations in waters under examination may be materially reduced by the following procedure.

First, extract the lead from the slightly ammoniacal water solution with an excess of dithizone. There is a tendency toward low results whenever a precipitate forms after adding the ammonia. Citrates will keep iron, aluminium, reasonable quantities of calcium phosphate and other calcium salts in solution; probably also magnesium salts, except magnesium ammonium phosphate. This phenomenon is due to the adsorption in lead of the ferric hydroxide or pectin type.

After the addition of the excess dithizone, shake with 50 ml. of 1 per cent  $\text{HNO}_3$  (free from oxides of nitrogen). The lead will go into the aqueous phase and the free dithizone will stay in the chloroform phase. Thus the lead is freed from the interfering effect of other metals, except bismuth, stannous tin and thal-

(Continued on page 216)

15 to 25 samples at one time. Once the evaporations are completed, the total time required for making 25 determinations is about one hour. The pH range of the samples is 10.5–10.8. The standards are slightly higher (approximately 11.3). Apparently a considerable amount of ammonia is lost during agitation in the open flask. Very little difference can be detected when the pH values extend as high as 12.0. It seems desirable, however, to have the pH for both samples and standards as nearly the same as possible.

Of the ions tested, Zn, Cu and Mn were the only ones found to interfere with the lead determinations. The interference in these cases is not particularly serious. Manganese does not influence the test unless present in quantities greater than 10 ppm. Zinc above 20 ppm. interferes. Such concentrations are not ordinarily encountered. The influence of zinc becomes appreciable at about 20 ppm., but the effect is slight below 50 ppm.

The influence can be lessened by using twice the amount of ammonia reagent recommended in the regular procedure. Copper does not interfere below 0.2 ppm., ~~the maximum limit permissible according to the "Drinking Water Standards" of the U. S. Treasury Dept.~~

Hum, none of which should be expected in water samples. To the entire 50 ml. of acid solution, or to an aliquot portion thereof made up to 50 ml. with 1 per cent HNO<sub>3</sub>, add 10 ml. of citrate-cyanide-ammonia solution (in this case, made up in such strength that the resulting mixture will have a pH of from 9.5 to 10). The lead may then be shaken out with standard dithione solution and the effect of most of the interfering materials will have been removed.

becomes bright yellow. The color which would ordinarily be indicative of lead is completely obscured. This is not an inconvenience, since the presence of so much copper is unlikely in a water used for drinking purposes.

#### EDITORIAL NOTE

This method is based upon research, as yet unpublished, by Prof. J. J. Hinman and Dr. J. E. Hedrick at the University of Iowa.

#### BIBLIOGRAPHY

- FISCHER, H. Ueber den Nachweis von Schwermetallen mit Hilfe von "Dithizon." *Zeit. Angew. Chem.*, 42, 1025 (1929).
- FISCHER, H., AND LEOPOLDI, G. Microchemical determination of heavy metals with dithizone. *Wiss. Ver. Siemens Konzern*, 12, 33 (1933).
- ROSS, J. R., AND LUCAS, C. C. A new method for the detection and estimation of minute amounts of lead. *Can. Med. Assn. J.*, 29, 649 (1933).
- WICHMAN, H. J., AND OTHERS. Methods for determination of lead in foods. *J. Assn. O. A. Chem.*, 17, 108 (1934).
- PUBLIC HEALTH SERVICE DRINKING WATER STANDARDS. *Pub. Health Repts.*, 61, 371 (1946).

#### 5. Phenols in Water

There are two colorimetric methods for the determination of phenol in water. Method A employs a 2,6-dibromoquinonechlorimide solution as an indicator. This indicator produces color in varying shades of blue. Method B employs a diazotized sulfanilic acid solution produces colors in varying shades of yellow. The methods are identical in sample preparation and calculation of results, but differ as to the preparation and comparison of color standards.

Both of these methods were described in Sec. IX, Appendix I, of the 8th Ed. of *Standard Methods for*

Corrected  
Error.  
CJDB.



*the Examination of Water and Sewage*. Since that edition was published, studies\* at the Laboratories of the Ohio State Department of Health have indicated the need for revision of those directions. R. D. Scott, Chief of the Ohio Laboratories recommended\* several changes, including the elimination of Method B.

Consistent with the intent of this Appendix, namely to furnish provisional technics that need further study, this section has been completely revised in accordance with Mr. Scott's recommendations. The original references for the methods as given in the 8th Ed., however, will be found at the end of this section.

### 1. Storing, Concentration, Preparation of Sample

1.1. Storing samples. Phenol in very weak solutions decomposes on standing and samples should be tested within 4 hours after the collection unless special precautions for storing have been taken. One g. of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  per liter will preserve the phenol fairly well for several days.

1.2. Distillation of sample. Determine the methyl orange alkalinity of the sample with 0.02 N HCl. To 220 ml. of the clear supernatant sample in the 500 ml. flask of a Pyrex all-glass still (Pyrex No. 3360) add just enough 0.1 N HCl to neutralize the alkalinity as previously determined.

Connect the flask to the condenser and distill slowly into a 200 ml. volumetric (glass stoppered) flask, exactly to the mark. Stopper and mix.

The error in the volume ratio (220 to 200) is practically balanced by the small proportion of phenols remaining in the distilling flask.

### 2. Standards

Coke oven wastes and streams receiving the wastes do not contain phenol alone, but contain variable proportions of cresols and phenol. The color of phenol with the Gibbs reagent may be described as blue-green; o-cresol as purple; m-cresol as blue; p-cresol produces no color. Satisfactory comparisons cannot be made of the blue-green phenol and indicator color against the usual purple color of the water sample distillate plus indicator. In order to obtain any accuracy it is necessary to prepare standards which contain a mixture of o-cresol and phenol, for example, equal parts of each.

2.1. Preparation of standard phenol-o-cresol: Weigh exactly 1 g. of cp. reagent grade phenol crystals into a 1 liter volumetric flask, fill to mark with distilled water, stopper and shake until dissolved. Also weigh exactly 1 g. of o-cresol (Eastman Kodak P-81) crystals into a liter vol. flask. Add 12 ml. of 1 N sodium hydroxide and when dissolved, fill to mark with distilled water; shake to mix. These stock solutions should be kept under refrigeration. Phenol may be standardized with a bromate-bromide reagent; o-cresol as in Appendix I, Sec. 5, reference.

Prepare a 10 ppm. solution of phenol-o-cresol by adding 5 ml. of each of the above 1000 ppm. solutions to a liter volumetric flask (10 ml. total), fill to mark with distilled water

\* Private communication to the Joint Editorial Committee, from R. D. Scott, Chief of Laboratories, Ohio Dept. of Health, Columbus, Ohio, May 7, 1942 and July 19, 1945.

and shake to mix. This 10 ppm. phenols solution should be made fresh each day it is used.

Prepare from the 10 ppm. phenols solution a series of standards such as 0, 0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 ppm. by adding the calculated volume, for example 5 ml. of the 10 ppm. soln. for 0.5 ppm. phenols, to 100 ml. 6 inch Nessler jars. Fill to mark with double distilled water. Add 5 ml. of buffer solution.

(Theriault's buffer solutions: \* 3.1 g. boric acid,  $H_3BO_3$ . 3.5 g. potassium chloride, KCl. 32 ml. 1 N NaOH. Dilute to 1 liter with distilled water. Five ml. diluted with 100 ml. distilled water has pH 9.4.)

### 3. Reagents

3.1. Indicator. Dissolve 0.04 g. of dibromoquinonechlorimide (Eastman Kodak No. 2304) in 10 ml. of ethyl alcohol.

### 4. Procedure

To sample distillate, 100 ml., 50 ml. diluted to 100 ml. and 25 ml. or other aliquots diluted to 100 ml. with distilled water add 5 ml. of buffer solution.

Add 2 drops of indicator to each Nessler jar, mix, let stand overnight and compare sample colors with standards.

With high phenols contents, base report on the aliquot which is nearest to 0.3 ppm. phenols.

If the color tint of standards using equal parts of phenol and o-cresol does not simulate that of the samples, use another proportion.

### BIBLIOGRAPHY

- GIBBS, H. D. Indol phenol test. *J. Biol. Chem.*, 72, 649 (1927).  
 SCOTT, W. W. *Standard Methods of Chemical Analysis*. 4th ed., p. 1549. Van Nostrand (1927).  
 CLARK, W. M. *The Determination of Hydrogen Ions*. 3d ed., Williams & Wilkins (1928).  
 THERIAULT, E. J. Chemical aspects of stream pollution by phenols. *Ind. Eng. Chem.*, 21, 4, 343 (1929).  
 BAYLIS, J. R. Procedure for making quantitative phenol determinations. *W. Wks. and Sew.*, 79, 341 (1932).

### 6. Phenol in Polluted Streams and Trade Wastes

For estimation of phenols in quantities 10 to 30 ppm., the titrimetric method of examination of brominated distilled samples is offered.

After suitable preliminary purification treatment an aliquot portion estimated to contain about 0.02 g. phenol is treated with potassium bromide solution and acidified. After temperature adjustment to 25° C. potassium bromate solution is added and after 1 hour's standing, potassium iodide solution. The liberated iodine is titrated with thiosulfate solution. A blank is run in parallel and phenol estimated by the difference in the two titrations.

### BIBLIOGRAPHY

- SCOTT, R. D. Application of a bromine method in determination of phenol and cresols. *Ind. Eng. Chem., Anal. Ed.*, 3, 67 (1931).

### 7. Volatile Acids in Digesting Sludges

To a sample of 50 ml. or more, add 2.5 ml. of concd.  $H_2SO_4$  and dilute to 200 ml. Distill off 150 ml. at the rate of 4 to 5 ml. per minute and titrate the distillate with 0.116 N NaOH.

\* *Ind. Eng. Chem.*, Apr., 1929.

using phenolphthalein as the indicator. The end point is the first pink coloration which disappears on standing in short time due to  $\text{CO}_2$ . Titration at  $95^\circ \text{C}$ . produces a stable end point.

The method is quite empirical and is not intended for accurate work. A factor of 0.7 is used to represent the amount of acid in the distillate. This factor has been found to vary between 0.68 and 0.85, depending on the nature of the acids and the rate of distillation.

Calculation:

ppm. volatile acid as acetic acid = ml. of  $0.116 \text{ N NaOH} \times [10,000 \div \text{ml. of sample}]$ .

If  $0.1 \text{ N NaOH}$  is used:

ppm. = ml. of  $0.100 \text{ N NaOH} \times [8,580 \div \text{ml. of sample}]$ .

## BIBLIOGRAPHY

*Illinois State Water Survey Bull. No. 30*, page 76 (1930).

## 8. Oxygen Demand and Activity of Activated Sludge

These tests are proposed to aid in control of operation of the activated sludge process. The extent to which activated sludge is loaded with organic matter is indicated by the amount of oxygen required by a given amount of sludge during a specified period of time. The relative oxidizing activity of an activated sludge may be obtained by determining the increase in the oxygen required by a given amount of sludge dosed with a standard amount of food.

These tests must be carried out with special equipment designed for the purpose. Several forms of apparatus

have been described, each having some advantages, any one of which may be employed.

A number of small compressors or air pumps are available, such as refrigerator compressors or paint spray pumps, but pumps employing oil in the pump mechanism should be avoided because of the absorption of oxygen by the oil.

These tests should preferably be carried out at constant temperature by maintaining the aerating sludge in a carefully controlled water bath or incubator.

To permit manometric measurement of the oxygen utilized provision is made for the removal of the carbon dioxide by sodium hydroxide. The vessel containing the sodium hydroxide should be provided with a distillation trap (similar to those used in free ammonia distillations) to prevent mechanical transfer of the alkali to the aeration mixture. A diagrammatic scheme of the apparatus is shown in Fig. 19. Air is pumped by means of the pump "B" through the caustic chamber "E," thence through the aeration chamber "A" and back to the pump through a surge chamber "C." The gas burette "D" is connected to the aeration system as shown in the diagram.

### 1. Reagents

1.1. Sodium hydroxide solution for  $\text{CO}_2$  absorption. Prepare a 10 per cent solution of cp.  $\text{NaOH}$ .

1.2. Peptone solution for activity test. Dissolve 20 g. of peptone in one liter of distilled water and dispense in 10 ml. quantities, into individual containers. The individual contain-



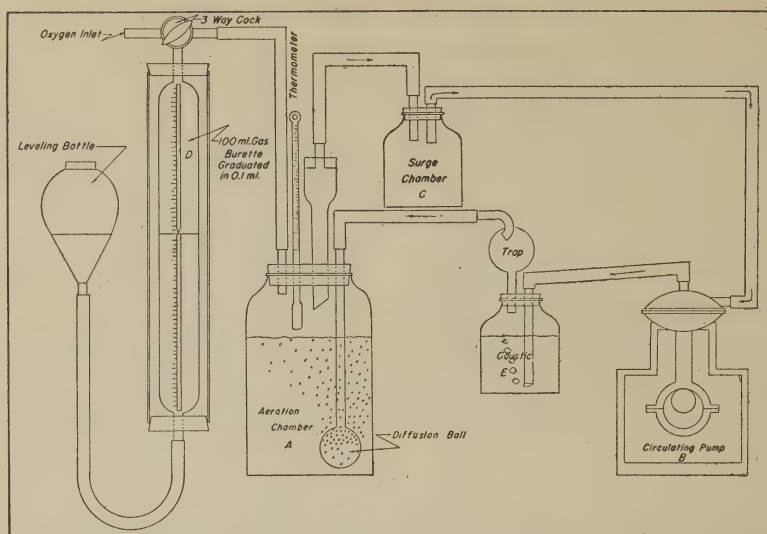


FIG. 19. SCHEMATIC DIAGRAM OF APPARATUS FOR THE DETERMINATION OF OXYGEN DEMAND OF ACTIVATED SLUDGE.

ers are stoppered with cotton and autoclaved at 15 lb. pressure for 15 minutes after which they may be stored in a refrigerator until used.

## 2. Procedure

### 2.1. . Oxygen demand of sludge.

As a routine test for control purposes in operating an activated sludge plant, the sludge to be tested is obtained from the effluent end of an aeration unit. This mixed liquor is immediately concentrated by settling and the supernatant is withdrawn to leave a sludge concentration of at least 5000 ppm. (0.50 per cent). The concentrated sludge is then aerated until the actual suspended solids have been determined (approximately 45 minutes), then the sludge is diluted with plant effluent until it contains exactly 5000 ppm. of solids. One liter of this sludge which has been brought to 20° C. is then put into the aeration chamber of the machine and about 60

ml. of the sodium hydroxide is put into the carbon dioxide trap and the system is closed.

When a bulking sludge cannot be concentrated to 5000 ppm. of solids a two liter portion containing 2500 ppm. of solids or a three liter portion containing 1667 ppm. should be used in the aeration chamber.

After the system is closed the aeration pump is started and the sludge demand is obtained by taking readings on the burette at 5 minute intervals. The quantities of oxygen used are measured by decreases in volume of gas in the gas burette. The quantity of oxygen used is converted to ppm. of oxygen per hour using the volume of sludge in the aeration chamber, the temperature and barometric pressure of the air as a basis of the calculation. The sludge demand figure is usually obtained by averaging the rates obtained per hour for the first three five minute periods.

To maintain a satisfactory sludge, the demand figures should not vary materially from day to day.

2.2. Sludge activity. To obtain the sludge activity, the aeration chamber is opened, after the sludge demand data are obtained, and 10 ml. of sterile peptone solution added to the sludge. Two minutes is then allowed for mixing of the synthetic sewage with the sludge before the system is again closed. Readings are then taken on the burette at five minute intervals for as long as it is deemed necessary. The maximum amount of oxygen is used in the first or second five minute period. This maximum reading less the sludge demand is the activity of the sludge and is also expressed in ppm. per hour. Additional information can be obtained by studying the data plotted against time. The shape of the curve indicates the quality of the sludge.

#### BIBLIOGRAPHY

BLOODGOOD, DON E. *Sew. Works J.*, 10, 27 (1938); 10, 927 (1938); 16, 913 (1944).

### 9. Determination of Grease in Sewage Sludge

#### A. WET EXTRACTION METHOD

##### 1. Reagents

- 1.1. Sulfuric acid cp. 1:1 solution.
- 1.2. Mixed hexanes as sold by Phillips Petroleum Corp., Bartlesville, Okla.

##### 2. Procedure

Any variations of detail which seem reasonable to an experienced chemist and which are carried out with due attention to the requirements of quantitative analytical technique are per-

missible. The results of the wet method are not appreciably influenced by minor modifications of procedure.

Compliance with the general directions leads to certain difficulties, notably that of separating the solvent after shaking. Methods which have been devised to accomplish this are set forth in the following suggested procedure suitable for all types of sewage, sludges, and other fluid materials.

2.1. Amount of sample. The amount of sample required for a test varies with the nature of the waste. It is generally desirable that the yield of grease be between 50 mg. and 500 mg. Sewage samples of 800 ml. to 2 liters have been found convenient, but where heterogeneity of the material makes large samples desirable, volumes as large as 4 liters may easily be analyzed by use of vessels of suitable size. In the case of sewage sludges, samples containing 1.0 to 5.0 g. of dry solids are usually satisfactory, but samples with 18 g. of dry solids have been analyzed without difficulty.

It is generally desirable that the sample be delivered in a glass-stoppered bottle containing the amount of material suitable for one analysis. In this way any grease adhering to the vessel may be washed into succeeding apparatus.

2.2. Acidification. Acidify the sample, using 5 ml. of 1:1  $H_2SO_4$ . (For samples larger than 1 liter, or for very alkaline materials, appropriately larger amounts of acid should be used.)

Transfer to a separatory funnel of

suitable size having a standard taper ground mouth. (In the case of samples too large for available funnels, the extraction may be carried out in bottles or other glass-stoppered apparatus.)

If the entire contents of the sample vessel are being analyzed, the container should be rinsed out with a little solvent, adding the rinsings to the funnel. Add to the funnel 75 to 150 ml. of solvent which has been distilled in all-glass apparatus. Use proportionally larger amounts for samples larger than 1 liter.

2.3. Extraction. Shake the funnel vigorously for at least two minutes. Very thick sludges should be thinned with water to facilitate this extraction step.

2.4. Refluxing. Connect the mouth of the funnel to a vertical condenser having a standard-taper ground-glass connection, and lower the assembly into a water bath maintained at a temperature of 90 to 100° C. (Cork connections may be substituted for ground-glass joints with introduction of scarcely appreciable errors.)

Reflux briskly until the emulsoid mixture has separated into its component parts. This usually requires from fifteen to thirty minutes. It is desirable that the ebullition be principally from the top of the water layer. If the boiling occurs from the bottom of the funnel, the emulsion may not break.

2.5. Removal of solvent. Disconnect the funnel and cool for five or ten minutes, preferably with water. Set up a filter over a weighed conical flask of 125 to 250 ml. capacity. Transfer the clear solvent solution to

the filter, using a 25 ml. pipette fitted with a 30 ml. rubber bulb. In order to avoid spilling drops of the solvent solution, the mouth of the separatory funnel should be held over the filter. Solvent must not be drawn into the rubber bulb.

To facilitate complete removal of the solvent layer, the water may be withdrawn from the bottom of the funnel, and then returned to the funnel after the solvent layer has been removed.

2.6. Repeat extraction and refluxing. Add 50 to 100 ml. of solvent to the funnel and repeat operations 2.3 to 2.5. If desired, all or part of the water may be discarded after the second extraction, since the remaining grease will be almost entirely in sludge solids and residual solvent. As many subsequent extractions are made as required to accomplish practically complete removal of the grease. For sewage, three or four extractions are sufficient, but sludges may require five or six extractions.

After all solvent layers have been transferred to the flask, the filter is washed thoroughly with fresh solvent.

2.7. Separation of solvent. Separation of the solvent from the fatty matter must be effected by a source of heat which cannot rise above 100° C. Either a water or steam bath is convenient. The flask may be connected to a condenser for recovery of the solvent, using ground-glass connections.

2.8. Drying and weighing of grease. When no more liquid solvent is visibly present, a jet of dry air or gas is introduced into the flask while it is still on the heating bath. This



serves to displace the heavy vapor. The amount of air or gas used should be about ten times the volume of the flask. Use of larger volumes increases errors due to vaporization of grease.

The flask is placed in a drying oven at 100° to 103° C. for 15 minutes, transferred to a desiccator and weighed when cool. In order to minimize absorption of oxygen, it is desirable that the time in the desiccator should not exceed three hours.

As a check on the completeness of the drying of the grease, reheat the flask for ten minutes and reweigh. If the grease does not lose more than two per cent of its weight as a result of this reheating, the first weighing should be considered correct.

Pomeroy and Wakeman also propose a variation of the above procedure for sewages which do not form emulsions.

## B. EXTRACTION OF COAGULATED GREASE

The following technic of Eliassen and Schulhoff consists of lime and alum coagulation, filtration on asbestos, acidification and fluffing of the asbestos, and extraction of the asbestos with petroleum ether for 3 to 4 hours.

### 1. Reagents

- 1.1. Petroleum ether.
- 1.2. Asbestos.
- 1.3. Calcium hydroxide—10 per cent suspension.
- 1.4. Alum—10 per cent solution.
- 1.5. Hydrochloric acid—1:1 solution.

### 2. Procedure

2.1. Coagulation of grease. Add 1.5 ml. of 10 per cent lime solution to at least 1 liter of sewage. Mix completely and add 4 ml. of 10 per cent alum solution. Mix thoroughly but gently and allow to settle for 1 hour. (The amount of lime may be varied but in all cases add enough to leave a residual carbonate alkalinity.)

Siphon off the supernatant liquor using care that neither the settled nor the floating grease be disturbed.

2.2. Preparation of crucible. Fold an 11 cm. piece of filter paper so that it lines the interior of a 25 ml. Gooch crucible to within 0.6 cm. of the top.

Add an asbestos suspension to the crucible and apply vacuum to produce a thick mat.

Add 0.75 g. of short fibered asbestos to the flocc (precipitated grease), mix well and pour into Gooch crucible. Apply vacuum until solids are partially dried.

2.3. Acidification. Remove crucible to shallow dish or beaker. Add 3 to 4 ml. of 1:1 HCl soln. slowly to the exposed surfaces of paper and mat. As the mat begins to fluff due to liberated carbon dioxide from the residual carbonate, place the dish in a 103° C. oven to aid in the evolution of CO<sub>2</sub> and consequent fluffing. Return acid through the crucible, then thoroughly dry crucible and contents in oven for 30 minutes.

2.4. Extraction. Wrap the crucible bottom and sides with aluminum foil and fill the crucible with glass beads to within 0.3 cm. of the top. Place the crucible in a weighed glass flask of a Bailey-Walker extraction

unit and extract for three to four hours with petroleum ether.

2.5. Drying and weighing. After extraction, remove the crucible from the flask. Drive off the petroleum ether in a drying oven at 103° C. for 15 minutes. Weigh the flask and residue and subtract the original weight of the flask to obtain the weight of the grease.

#### BIBLIOGRAPHY

POMEROY, RICHARD, AND WAKEMAN, C. M. Determination of grease in sewage, sludges and industrial wastes. *Ind. Eng. Chem., Anal. Ed.*, 13, 795 (1941).

ELIASSEN, ROLF, AND SCHULOFF, H. B. Grease extraction from sewage and sludge. *Sew. Works J.*, 15, 491 (1943).

#### 10. pH of Sewage and Sludge Colorimetrically

If the glass electrode is not available fairly satisfactory results may be obtained by colorimetric procedures using the sulfaphthalein indicators and the buffer solutions as recommended by Clark.

Commercial indicator sets are available and if frequently checked against standard buffer solutions will give satisfactory results, providing the indicator is purchased from the manufacturer of the set. Procedures are furnished with these commercial sets and should be carefully followed. The colorimetric comparison may be accomplished by using a photoelectric photometer, the proper light filter and a standardized curve obtained with standard buffers and indicators.

#### 1. pH of Thick Sludges

It is often difficult, with sewage sludges, to obtain a sufficiently clear

supernatant liquor for colorimetric work, and the dilution procedure is often unsatisfactory because the buffer value of the sludge will not stand dilution. A clear sample for colorimetric pH may be easily and accurately obtained by diffusion through dialyzer parchment paper or cellophane.

Diffusion cells are made by cutting off the bottoms of 120 ml. (4 ounce) glass bottles and covering the opening with parchment paper by means of a rubber band. The inside of the cell is then filled with aerated distilled water (pH 6.9 to 7.0) and suspended in a large beaker of sludge, the two liquid levels being about equal. Within 15 minutes the distilled water has reached equilibrium with the sludge, and a perfectly clear solution is available on which to run colorimetric pH determinations. Dialyzer tubing may be used instead of the cell, although the cell is more easily handled than the tubing.

#### BIBLIOGRAPHY

CLARK, W. M. *The Determination of Hydrogen ions*. 3rd Ed. Williams & Wilkins, Baltimore (1928).

HATFIELD, WILLIAM D., AND PHILLIPS, GEORGE E. Modified photoelectric photometer. *Ind. Eng. Chem., Anal. Ed.*, 13, 430 (1941).

#### 11. Organic Carbon in Sewage

According to F. W. Gilreas of the New York Health Dept. Laboratories, experience has indicated that a modification, as given below, of the details of the original method leads to simplicity in operation and greater accuracy in the determination:

### 1. Apparatus

The apparatus assembly is shown in Fig. 20. Air is passed through a wash bottle (A) containing saturated sodium hydroxide and glass beads, through an absorption tower (B) containing moist soda lime, and is bubbled through the reaction vessel (C). It is then passed through a gas washing bottle containing acid potassium iodide (D), a Venier zinc tube containing sulfuric acid (E), and a Venier zinc tube containing dehydrite (F) to remove evolved chlorine and moisture. The carbon dioxide is absorbed in a weighed Midvale absorption bulb (G) containing ascarite. The bulb is protected from moisture by a Venier zinc tube containing dehydrite (H) at the end of the chain.

### 2. Reagents

2.1. Chromic acid. Dissolve 340 g. of chromic oxide in 400 ml. of hot carbon-dioxide-free water and make up to 1 liter with 85 per cent phosphoric acid.

2.2. Sulfuric-phosphoric acid. Use equal volumes of concd.  $\text{H}_2\text{SO}_4$  and 85 per cent  $\text{H}_3\text{PO}_4$ .

2.3. Sulfuric acid. Heat concd.  $\text{H}_2\text{SO}_4$  in a Kjeldahl flask for 2 hours.

2.4. Acid potassium iodide. Dissolve 35 g. of KI in 25 ml. of distilled water and add 2 ml. of 1 N  $\text{H}_2\text{SO}_4$ .

### 3. Procedure

Draw air through apparatus at the rate of 100 to 200 bubbles per minute for about 30 minutes. Close all stop-cocks, remove absorption bulb and weigh. With the vacuum line closed,

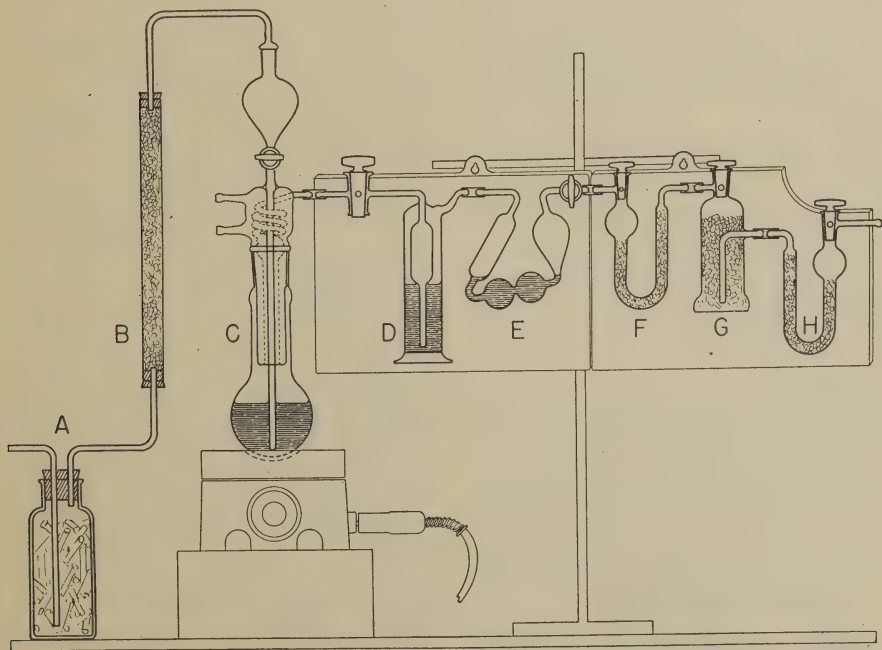


FIG. 20. APPARATUS FOR DETERMINING ORGANIC CARBON IN SEWAGE.



replace bulb and open stopcocks. Add to the reaction chamber through the separatory funnel a sample that contains from 10 to 40 mg. of carbon, then 10 ml. of chromic acid, followed by 50 ml. of sulfuric-phosphoric acid and 2 ml. of  $H_2SO_4$  for each ml. of sample. Use just sufficient vacuum to draw the sample and reagents into the flask. Close opening into separatory funnel with connection to air scrubber. Turn on cooling water connection to condenser. Turn on electric heater and increase vacuum to 100 to 200 bubbles per minute. Run for 2 hours at a gentle boil. Then close all stopcocks, remove bulb, and weigh to determine total carbon.

Another sample from the same source is treated with a few drops of concd.  $H_2SO_4$  only and run one hour as before to determine the inorganic carbon.

A reagent blank is run with an equivalent volume of carbon-dioxide-free distilled water in place of the sample.

The efficiency of digestion and absorption is checked by using as sample a definite amount of potassium acid phthalate in distilled water.

Organic carbon = total carbon - (inorganic carbon + blank).

## BIBLIOGRAPHY

- MOHLMAN, F. W., AND EDWARDS, G. P. Determination of carbon in sewage. *Ind. Eng. Chem., Anal. Ed.*, 3, 119 (1931).  
 GILCREAS, F. W. Private Communication to the Joint Editorial Committee (1940).

## 12. Broths, Selective, for Primary or Parallel Planting

For the control of water filtration plant operation it is sometimes ad-

vantageous to use a parallel planting procedure in connection with the standard method for testing for the presence of members of the coliform group. This procedure consists in the simultaneous planting of portions of samples in standard lactose broth and in some other liquid medium which has selective properties. The results derived from such a procedure must be interpreted in the light of experience in the particular plant involved. They must not be used for securing official estimates of the density of coliform organisms.

Inasmuch as the methods of using and interpreting such procedures depend on a wide variety of circumstances, outlines of such methods are left to those who desire to use them. Typical liquid media that are more or less selective for coliform organisms are:

- A. Fuchsin Lactose Broth.
- B. Brilliant-Green Lactose Bile (see Part IV, Sec. 3, K, page 188).
- C. Methylene-Blue Erythrosine Bromocresol-Purple Broth.
- D. Formate Ricinoleate Broth (see Part IV, Sec. 3, L, page 188).
- E. Crystal Violet Broth.
- F. Eijkman Broth.
- G. EC Medium.

These media are presumed to be reasonably specific for coliform group organisms in their growth-producing qualities. As a rule, however, they are known to inhibit the coliform group in varying degrees and thus should not be substituted for lactose broth in primary planting. Incubation is usually at  $35^\circ$  to  $37^\circ$  C. for  $48 \pm 3$  hours. Gas production (and color changes in certain cases) is

taken as evidence of the presence of members of the coliform group.

For preparation of the media listed above but not included in Part IV, Sec. 3, the references noted in the bibliography should be consulted. When quantities greater than 1 ml. of sample are planted in any of these media, care should be exercised to adjust the strength of the medium so that the concentrations of the ingredients in the mixtures of medium and sample will not be appreciably different from those specified in the basic formulae.

#### BIBLIOGRAPHY

- DOMINICK, J. F., AND LAUTER, C. J. Methylene blue and brom cresol purple in differentiating bacteria of the colon-serogenes group. *J. Amer. W. W. Assn.*, 21, 1067 (1929).
- SALLE, A. J. A system for the bacteriological examination of water. *J. Bact.*, 20, 381 (1930).
- RITTER, C. The presumptive test in water analysis. *J. Amer. W. W. Assn.*, 24, 413 (1932).
- PERRY, C. A., AND HAJNA, A. A. A modified Eijkman medium. *J. Bact.*, 26, 419 (1933).
- HAJNA, A. A., AND PERRY, C. A. A comparison of the Eijkman test with other tests for determining *E. coli*. *J. Bact.*, 30, 479 (1935).
- HAJNA, A. A., AND PERRY, C. A. Comparative study of presumptive and confirmative media for bacteria of the coliform group and for fecal streptococci. *Am. J. Pub. Health*, 33, 550 (1943).
- PERRY, C. A., AND HAJNA, A. A. Further evaluation of EC medium for the isolation of coliform bacteria and *Escherichia coli*. *Am. J. Pub. Health*, 34, 735 (1944).

### 13. Selective Agar Media for Differentiation in the Coliform Group

In the outline of procedures for bacteriological examination of water in Part IV, Sec. 9, A, 7, page 193 and

Sec. 12, page 207 it is emphasized that primary plantings in lactose broth should not be used as the first step in differentiation of members of the coliform group.

Tests for the relative density of *E. coli*, *E. freundii* (intermediate) or *A. aerogenes* in a sample shall be made by planting a sufficient quantity of the sample in a medium which becomes solid at incubator temperature (35° to 37° C.) and on which the organisms of these species grow with distinct and differentiating characteristics.

Cyanide citrate agar, methylene-blue erythrosine bromocresol-purple agar, brilliant-green bile agar, eosin methylene-blue agar (modified) or bile salt agar may be used for this purpose.

Plates shall be poured in such numbers or having such density of colony growth as to produce not less than 10 colonies of the coliform group if the relative density of each species is to be recorded.

It should be remembered that the present state of knowledge regarding the relative growth-supporting powers of these media on the one hand, and lactose broth on the other, is not sufficient to justify the enumeration of absolute density of coliform group organisms by the pour plate method. The decimal dilution method, involving the use of primary lactose broth tubes, remains the accepted method of enumerating coliform density.

Samples plated in the media herein after listed should be incubated for the period indicated specifically for each medium. After recording an estimate of the various types of colo-

nies present, further differentiation may be made as outlined in Appendix I, Sec. 14, page 228.

Some of the media that may be used for differential plate counts are:

A. Bile Salt Agar.

B. Brilliant-Green Lactose Bile Agar.

C. Ferrocyaniide Citrate Agar.

D. Methylene-Blue Erythrosine Bromocresol-Purple Agar.

E. Eosin Methylene-Blue Agar (Levine modified).

Reference to the literature cited should be made for detailed information regarding the preparation and use of these media.

Various bile salt agars have been suggested for direct plating of water samples. The best known of this type of agar is probably MacConkey Agar as used in Britain, which employs European brands of bile salt mixtures. Somewhat similar media are a MacConkey Agar\* employing an American brand of bile salt mixture, Violet Red Bile Agar,\* and Desoxycholate Lactose Agar,† the dehydrated form and the ingredients of which can be obtained from American supply houses.

When using these bile salt media, it is advisable, after mixing the sample and medium in the plate and allowing it to harden, to pour a thin cover of the agar medium over all and allow this to harden. By proceeding in this manner, a minimum of surface colonies, which may present a non-characteristic appearance, is produced.

\* Obtainable from Difco Laboratories, Detroit, Mich.

† Obtainable from Baltimore Biological Laboratory, Baltimore, Md.

## BIBLIOGRAPHY

- MACCONKEY, A. T. Bile salt media and their advantages. *J. Hyg.*, 8, 322 (1908).  
 SALLE, A. J. A system for the bacteriological examination of water. *J. Bact.*, 20, 381 (1930).  
 TONNEY, F. O., AND NOBLE, R. E. An improved ferrocyaniide-citrate agar for direct enumeration of colon-aerogenes organisms. *J. Amer. W. W. Assn.*, 23, 1202 (1931).  
 TONNEY, F. O., AND NOBLE, R. E. The interpretation of direct differential counts of colon-aerogenes organisms in well waters. *J. Bact.*, 23, 473 (1932).  
 NOBLE, R. E., AND TONNEY, F. O. A solid brilliant green lactose bile medium for direct plating. *J. Amer. W. W. Assn.*, 27, 108 (1935).  
 SCHULHOFF, H. B., AND HEUKELEKIAN, H. A direct plating method for the determination of the potability of water. *J. Amer. W. W. Assn.*, 28, 1963 (1936).  
 The Bacteriological Examination of Water Supplies. *Public Health Report No. 71*, 1939 (revised). Ministry of Health, London.

## 14. Differentiation—Coliform Group Organisms

### A. CULTURE PURIFICATION

It is well known that the accuracy of the Completed Test and differential tests is at times impaired by failure to purify cultures adequately. Care must be taken to make sure that cultures do not consist of mixtures of species. Any reasonable doubt in this regard should be eliminated by transfer to liquid media and replating, repeatedly, if necessary.

Variation in organisms of the coliform group, particularly the "unstable" variation characteristic of the *mutabile* type, is occasionally encountered. It is advisable, therefore, when attempting purification of cultures, to be on the lookout for this phenomenon. An apparent mixture of organisms in a culture may, in



reality, consist of a single strain that is showing variation.

## B. DIFFERENTIATION OF MEMBERS OF THE COLIFORM GROUP

For a satisfactory differentiation of the coliform group into the *Escherichia coli*, *Aerobacter aerogenes* and *Escherichia freundii* (or intermediate) species, four tests (indol, methyl red, Voges-Proskauer and sodium citrate) are commonly required. These four tests are tentatively recommended for such differential determination. If additional tests are added to these, the real and apparent variety of strains within the coliform group may be correspondingly increased as has been done in the classical systems of MacConkey, Clemesha and Levine. Although such intensive differentiation may be desirable from a research standpoint, it does not appear to be warranted in routine water work.

A simplified grouping of the reaction combinations and their suggested interpretations are shown below. It must be remembered, however, that all types of coliform organ-

isms may occur in feces. Although *E. coli* will nearly always be found in fresh pollution derived from several sources, some other type or types of coliform organisms, not accompanied by *E. coli*, may occasionally be found in fresh pollution from a single source; furthermore, the presence of *E. coli* may not be indicative of fresh pollution, particularly if the pollution derive from a single source, such as a person harboring in his feces *E. coli* alone.

On the other hand, there is little or no evidence that coliform bacteria multiply on fresh grasses or grains; nor is there evidence that they multiply in soil. Consequently, it is at least debatable whether grasses, grains and soils can be considered normal habitats of any of the coliform organisms, except perhaps occasional rapidly fermenting *Erwinia*, *Serratia*, or slime-producing forms which apparently do not occur in natural waters in significant numbers. Much more study of a quantitative character is needed before the practical value of routine differentiation in the coliform group can be demonstrated.

TABLE 20.—COLIFORM GROUP—REACTION CLASSIFICATION

	Indole	Methyl Red	Voges-Proskauer	Citrate	Commonly designated source
<i>Escherichia coli</i>					
Variety I.....	+	+	—	—	fecal
Variety II.....	—	+	—	—	fecal
<i>Escherichia freundii</i> (Intermediates)					
Variety I.....	—	+	—	±	non-fecal
Variety II.....	+	+	—	+	non-fecal
<i>Aerobacter aerogenes</i>					
Variety I.....	—	—	+	±	non-fecal
Variety II.....	—	—	+	+	non-fecal

Note: Some authors (Levine, and Stuart, *et al.*) suggest elimination of the Methyl Red test, or substitution of the Eijkman test, contending that the former furnishes no information not supplied by the Voges-Proskauer test and that the latter sharply differentiates *E. coli* from *E. freundii* and *A. aerogenes*.

It is well to keep in mind, however, the possibility of occasional multiplication of coliform organisms on leather washers, wood, swimming pool ropes, jute packing, in pipes, etc. In fact, differentiation of coliform types finds one of its most practical applications in the study of unexpected coliform densities that may be explained by multiplication on or in such organic materials. The presence of a large number of coliform organisms of the same type in water from a well or spring, or from a single tap on a distribution system, for example, is very suggestive of such multiplication.

#### C. SLOW OR WEAK LACTOSE FERMENTING ORGANISMS

The significance to be attributed to the presence of slow or weak lactose-fermenting coliform and related organisms in water supplies has been the subject of considerable study in recent years. Although it is possible that further experience will indicate differentiation in this group to be of practical value, all coliform organisms should, for the present, be considered of like significance when found in the course of routine examinations of water supplies.

#### D. INDOLE DIFFERENTIAL TEST

##### 1. *Reagents*

1.1. Tryptone broth. To 1 liter of distilled water add 10 g. of Bacto-tryptone (formerly tryptophane broth) and heat with stirring to obtain complete solution.

Distribute in 5 ml. portions into test tubes and sterilize in autoclave as in Part IV, Sec. 3, B, page 185.

1.2. Test reagent. Dissolve 5 g. of cp. paradimethyl-amino benzaldehyde in 75 ml. of amyl alcohol and add 25 ml. of coned. HCl. This reagent should have a yellow color.

Some brands of cp. amyl alcohol are not satisfactory. Laboratory reagent amyl alcohol is recommended. Some brands of paradimethyl-amino benzaldehyde are not satisfactory and some good brands become unsatisfactory on aging. Both amyl alcohol and benzaldehyde compound should be purchased in as small amounts as are consistent with the volume of work to be done.

##### 2. *Procedure*

Inoculate 5 ml. portions of medium. Incubate at 35° to 37° C. for  $24 \pm 2$  hours.

Add 0.2 to 0.3 ml. of the amyl alcohol indole reagent and shake. Let tube stand for about 10 minutes and observe the result.

A dark red color in the amyl alcohol surface layer constitutes a positive indole test; the original color of the amyl alcohol reagent, a negative test.

#### E. METHYL RED DIFFERENTIAL TEST

##### 1. *Reagents*

1.1. Peptone Medium.\* To 800 ml. of distilled water add 5 g. of Proteose-peptone, Difco, or Witte's Peptone (other peptones should not be substituted), 5 g. cp. dextrose, and 5 g. dipotassium hydrogen phosphate ( $K_2HPO_4$ ). A dilute solution of the  $K_2$ -

\* Use of M.R.-V.P. Medium (Difco dehydrated) with sterilization in the autoclave for 12 minutes at 15 pounds pressure is also recommended.

HPO<sub>4</sub> should give a distinct pink with phenolphthalein.

Heat over steam, with occasional stirring, for 20 minutes.

Filter through folded filter paper, cool at 20° C., and dilute to 1 liter with distilled water.

Distribute 10 ml. portions in sterilized test tubes.

Sterilize by the intermittent method for 20 minutes on three successive days.

1.2. Indicator solution. Dissolve 0.1 g. methyl red in 300 ml. alcohol and dilute to 500 ml. with distilled water.

## 2. Procedure

Inoculate 10 ml. portions of medium. Incubate at 30° C. for 5 days. To 5 ml. of the culture add 5 drops of methyl red indicator solution.

Record distinct red color as methyl red positive (+) and a distinct yellow color as methyl red negative (−) and intermediate as (?).

## F. VOGES-PROSKAUER DIFFERENTIAL TEST

### 1. Reagents

1.1. Medium. This test may be made on a 5 ml. portion of the medium inoculated for the methyl red test or on a separately inoculated tube of the same medium. The test should be made after 24 to 48 hours of incubation at 30° C.

1.2. Five per cent  $\alpha$ -naphthol in absolute alcohol.

1.3. Forty per cent KOH in water.

### 2. Procedure

To 1 ml. of culture add 0.6 ml. of 5 per cent  $\alpha$ -naphthol in absolute ethyl

alcohol and 0.2 ml. of 40 per cent KOH soln.

The development of a crimson to ruby color in the mixture from 2 to 4 hours after adding the reagents constitutes a positive test. Results should be read not later than 4 hours after addition of the reagents.

## G. SODIUM CITRATE DIFFERENTIAL TEST

### 1. Reagents

1.1. Medium. Dissolve 1.5 g. sodium ammonium phosphate (microcosmic salt), 1 g. potassium dihydrogen phosphate, 0.2 g. magnesium sulfate and 2.5 to 3.0 g. sodium citrate (crystals) in 1 liter of distilled water.

Distribute into test tubes in 5 ml. amounts and sterilize as in Part IV, Sec. 3, B, page 185.

### 2. Procedure

The inoculation into this medium shall be made with a needle or a standard loop. Inoculate lightly. A pipette should never be used, because of the danger of invalidating the result by introduction of nutrient material with the transfer. Incubate at 35° to 37° C. for 72 to 96 hours, and record visible growth as positive (+), no growth as negative (−).

## H. EIJKMAN DIFFERENTIAL TEST

The Eijkman test (see Appendix I, Sec. 12, page 226) has been frequently used as a test for determining the probable origin of coliform bacteria isolated from water samples. The great majority of *E. coli*, but only few *A. aerogenes* and *E. freundii* produce gas under the conditions of this test. Consequently, it is contended that a positive result (gas production) with



the Eijkman test constitutes, from a practical standpoint, an indication of the presence of "fecal" *E. coli*.

## 1. Reagents

1.1. Medium. Dissolve the following substances in distilled water and make up to 1 liter.

Tryptose .....	15.0 g.
Lactose .....	3.0 g.
Dipotassium phosphate .....	4.0 g.
Potassium dihydrogen phosphate ...	1.5 g.
Sodium chloride .....	5.0 g.

## 2. Procedure

Inoculate a fermentation tube of the medium with a pure coliform culture and incubate in a carefully regulated water jacketed incubator maintained at a temperature of  $45.5 \pm 0.2^\circ$  C. for 48 hours.

Production of gas constitutes a positive result.

## BIBLIOGRAPHY

- EIJKMAN, C. Die Gärungsprobe bei 46 C. als Hilfsmittel bei der Trinkwasseruntersuchung. *Zent. f. Bakt. Parasitenk., I, Orig.*, 37, 742 (1904).
- MACCONKEY, A. Lactose fermenting bacteria in feces. *J. Hyg.*, 5, 333 (1905).
- MACCONKEY, A. Further observations on the differentiation of lactose fermenting bacteria with special reference to those of intestinal origin. *J. Hyg.*, 5, 86 (1909).
- CLEMESHA, W. W. *The Bacteriology of Surface Waters in the Tropics*. Thacker, Spink & Co., Calcutta and London (1912).
- ROGERS, L. A., CLARK, W. M., AND DAVIS, B. J. The colon group of bacteria. *J. Infect. Dis.*, 14, 411 (1914).
- ROGERS, L. A., CLARK, W. M., AND EVANS, A. C. The characteristics of bacteria of the colon type found in bovine feces. *J. Infect. Dis.*, 15, 99 (1915).
- ROGERS, L. A., CLARK, W. M., AND EVANS, A. C. The characteristics of bacteria of the colon type occurring on grains. *J. Infect. Dis.*, 17, 137 (1915).
- CLARK, W. M. The final hydrogen-ion concentrations of cultures of *Bacillus coli*. *Science*, n. s. 42, 71 (1915).
- CLARK, W. M., AND LUBS, W. A. The differentiation of bacteria of the colon-aerogenes family by the use of indicators. *J. Infect. Dis.*, 17, 160 (1915).
- LEVINE, MAX. On the significance of the Voges-Proskauer reaction. *J. Bact.*, 1, 153 (1916).
- LEVINE, MAX. Bacteria fermenting lactose and their significance in water analysis. *Iowa State College Agr. & Mech. Arts Bull. No. 62*, Vol. 20, No. 31 (1921).
- KOSER, S. A. Correlation of citrate utilization by members of the colon-aerogenes group with other differential characteristics and with habitat. *J. Bact.*, 9, 59 (1924).
- KOSER, S. A. Differential tests for colon-aerogenes group in relation to sanitary quality of water. *J. Infect. Dis.*, 35, 14 (1924).
- MELLON, R. R. Studies in microbial heredity. II. The sexual cycle of *B. coli* in relation to the origin of variants with special reference to Neisser and Massini's *B. coli mutabile*. *J. Bact.*, 10, 579 (1925).
- KOSER, S. A. Cellobiose fermentation by the coli-aerogenes group. *J. Infect. Dis.*, 38, 505 (1926).
- KOVACS, N. A simplified method for detecting indol-formation by bacteria. *Ztschr. f. Immunitätsforsch., u. exper. Therap.*, 56, 311 (1928); *Chem. Abstr.*, 22, 3425.
- GREER, F. E., NOBLE, R. E., NYHAN, F. V., AND O'NEIL, A. E. The sanitary significance of lactose fermenting organisms not belonging to the *B. coli* group. *J. Infect. Dis.*, 42, 556 (1928).
- RUCHHOFF, C. C., KALLAS, J. G., CHINN, B., AND COULTER, E. W. Coli-aerogenes differentiation in water analysis. *J. Bact.*, 21, 407 (1930); 22, 125 (1931).
- LEAHY, H. W. Cotton guard rope in swimming pools as source of colon-aerogenes group. *J. Amer. W. W. Assn.*, 24, 1062 (1932).
- PERRY, C. A., AND HAJNA, A. A. A modified Eijkman medium. *J. Bact.*, 26, 419 (1933).
- CALDWELL, E. L., AND PARR, L. W. Pump infection under normal conditions in controlled experimental fields. *J. Amer. W. W. Assn.*, 25, 1107 (1933).
- RAPP, W. M., AND WEIR, PAUL. Cotton caulking yarn. *J. Amer. W. W. Assn.*, 26, 743 (1934).
- HAJNA, A. A., AND PERRY, C. A. A comparison of the Eijkman test with other tests for determining *E. coli*. *J. Bact.*, 30, 479 (1935).

BARRITT, M. W. The intensification of the Voges-Proskauer reaction by the addition of alpha-naphthol. *J. Path. & Bact.*, 42, 441 (1936).

PARR, LELAND W. Viability of coli-aerogenes organisms in culture and in various environments. *J. Infect. Dis.*, 60, 291 (1937).

FOOTE, H. B. The possible effects of wild animals on the bacterial pollution of water. *J. Amer. W. W. Assn.*, 24, 72 (1937).

PARR, LELAND W. Organisms involved in the pollution of water from long stored feces. *Am. J. Pub. Health*, 28, 445 (1938).

STUART, C. A., GRIFFIN, A. M., AND BAKER, M. E. Relationships of coliform organisms. *J. Bact.*, 36, 391 (1938).

PARR, LELAND W. The occurrence and succession of coliform organisms in human feces. *Am. J. Hyg.*, 27, 67 (1938).

PARR, LELAND W. Coliform bacteria. *Bact. Rev.*, 3, 1 (1939).

VAUGHN, R., MITCHELL, N. B., AND LEVINE, MAX. The Voges-Proskauer and methyl red reactions in the coli-aerogenes group. *J. Amer. W. W. Assn.*, 31, 993 (1939).

HAJNA, A. A., AND PERRY, C. A. Optimum temperature for differentiation of *Escherichia coli* from other coliform bacteria. *J. Bact.*, 38, 275 (1939).

MCCRADY, M. H. Slow lactose fermenters in water analysis. *Am. J. Public Health*, 29, 261 (1939).

HOWARD, N. J. Bacterial depreciation of water quality in distribution systems. *J. Amer. W. W. Assn.*, 32, 1501 (1940).

STUART, C. A., MICKLE, F. L., AND BORMAN, E. K. Suggested grouping of slow lactose fermenting organisms. *Am. J. Pub. Health*, 30, 499 (1940).

LEVINE, MAX. Determination and characterization of coliform bacteria from chlorinated waters. *Am. J. Pub. Health*, 31, 351 (1941).

BORMAN, E. K., ROBINSON, E. D., AND STUART, C. A. A study of standard methods for the detection of coliform organisms in raw and treated waters. *Am. J. Pub. Health*, 31, 557 (1941).

TAYLOR, C. B. The ecology and significance of the different types of coliform bacteria found in water. *J. Hyg.*, 42, 23 (1942).

STUART, C. A., ZIMMERMAN, ALICE, BAKER, MURIEL, AND RUSTIGAN, ROBERT. Eijkman relationships of the coliform and related bacteria. *J. Bact.*, 43, 557 (1942).

PARR, L. W., AND FRIEDLANDER, H. Studies on aberrant coliform bacteria. *Am. J. Pub. Health*, 32, 381 (1942).

STUART, C. A., WHEELER, K. M., RUSTIGAN, R., AND ZIMMERMAN, A. Biochemical and antigenic relationships of the paracolon bacteria. *J. Bact.*, 45, 101 (1943).

BORMAN, E. K., STUART, C. A., AND WHEELER, K. M. Taxonomy of the family *Enterobacteriaceae*. *J. Bact.*, 48, 351 (1944).

SANBORN, J. R. Slime-producing coliform and coliform-like bacteria. *J. Bact.*, 48, 211 (1944).

## 15. British Practice—Bacteriological Examination

In 1934, there was published a manual entitled "The Bacteriological Examination of Water Supplies" as Report No. 71 in the British Ministry of Health Series. It constituted for all practical purposes, the first British equivalent of the bacteriological part (Part IV) of *Standard Methods for the Examination of Water and Sewage*.

NOTE: The material beginning with paragraph A on page 234 to page 245 is quoted from this report.

A revised edition of Report No. 71 \* appeared in 1939. Inasmuch as its content is of interest to all persons using this text, extracts therefrom, reproduced herewith by permission granted by the Controller of His Britannic Majesty's Stationery Office, have been chosen for the purpose of indicating the English view of the rationale of the bacteriological examination of water, of the types of examination necessary in various circumstances and of the interpretation to be given the results obtained. For details of the methods of performing the various bacteriological tests, the complete Report should be consulted.

\* The Bacteriological Examination of Water Supplies, No. 71 (Revised Edition) of Reports on Public Health and Medical Subjects, Ministry of Health, 59 pages. Published by His Majesty's Stationery Office, 1939.

#### A. THE RATIONALE OF THE BACTERIOLOGICAL ANALYSIS OF WATER

The direct search for the presence of specific pathogenic bacteria in water is unlikely to prove of value for routine control purposes. There are several reasons for this. If a given supply receives a single contamination, for example from a typhoid carrier, it will probably be about a fortnight before a case of typhoid develops and another week or even longer before it is diagnosed and reported to the Medical Officer of Health. Since typhoid bacilli die out fairly rapidly in water it is improbable that the bacteriologist, after this lapse of time, will succeed in demonstrating their presence. In addition, the organisms usually gain access to the water in only very small numbers, and the technical difficulties of isolating small numbers of typhoid bacilli from water contaminated with excretal material are very great. If, of course, the contamination is repeated or continuous, then the chance of finding the bacillus is rather greater, but in practice suspicion is almost invariably thrown on the water as the result of epidemiological rather than of bacteriological inquiry.

The earlier workers, realizing the difficulty of demonstrating directly the presence of pathogenic organisms, adopted an indirect approach to the solution of the problem. It was suggested that the water should be examined for evidence of excretal or sewage pollution, the assumption being that if pollution of this type did occur, the water must be regarded as potentially dangerous. Experience

has shown that this reasoning was sound in practice.

During the last century methods were evolved for the detection of organic matter in water by chemical means, and further methods were elaborated, depending on the estimation of free and albuminoid ammonia, chlorine, nitrates and nitrites, for distinguishing between organic matter of animal and vegetable origin. In this way considerable progress was made, but in spite of the large amount of work carried out it was found that these tests were not sufficiently delicate or specific for the detection of minor degrees of sewage contamination.

With the rise of bacteriology, efforts were made to supplement the chemical analysis. These consisted at first of approximate estimations of the numbers of bacteria capable of growing at atmospheric and at body temperatures. The greater the number of colonies developing at atmospheric temperature, the greater presumably was the amount of organic matter in the water; and the greater the number of colonies developing at body temperature the more likely were organisms of sewage and parasitic origin to be present. These tests, however, had little advantage over the methods evolved by the chemists.

Attention was therefore directed to the demonstration of bacterial species of known excretal origin, particularly organisms of the coliform group, faecal streptococci, and *Clostridium welchii*. Since these organisms are constantly present in the human intestine, usually in numbers greatly



exceeding those of pathogenic intestinal bacilli, and since their death rate in water is rather slower than that of organisms of the enteric group, it follows that whenever typhoid or paratyphoid bacilli, for example, gain access to a water supply through excretal pollution they are always accompanied by the natural organisms inhabiting the intestine.

The finding of coliform bacilli in water shows, therefore, that recent excretal pollution has probably occurred, and, though not constituting of itself conclusive evidence of danger, is nevertheless sufficient to indicate that the water is potentially dangerous. Attention became more and more directed to the coliform group rather than to faecal streptococci and *Cl. welchii*, partly because of the greater ease with which coliform bacilli could be demonstrated and partly because *Cl. welchii* was found to survive considerably longer in water than members of the coliform and typhoid-paratyphoid groups.

More recent work has rendered it evident that the coliform bacilli constitute a group of organisms of diverse origin. Some of them, such as *Bact. coli*, have their normal habitat in the human and animal intestine, whereas others, such as *Bact. aerogenes*, *Bact. cloacae*, the so-called intermediate group, and certain irregular types, appear to be distributed mainly in vegetable matter and soil.

It is true that these non-parasitic types may gain access to the intestine by means of food and drink and may survive in it for a time, but the available evidence suggests that they are seldom present in numbers equal to

those of the typical *Bact. coli*. So far as we know, *Bact. coli* does not lead a saprophytic existence, except in certain special conditions, and the presence of this organism in water can be regarded as almost undoubted evidence of recent excretal pollution of human or animal origin.

Since *Bact. coli* can be detected in numbers as small as one in 100 ml. of water, the demonstration of this organism affords far and away the most delicate and specific test at our disposal for proving that excretal contamination has recently occurred. For this reason very careful estimation has to be made of the numbers of *Bact. coli* before deciding whether the pollution has been severe enough to render the water potentially dangerous, i.e., the whole essence of the test is quantitative.

It will be seen that the tendency of water analysis has been to pass from the more crude and less discriminative methods of chemical examination to the more delicate and more specific methods of bacteriological examination. At the present time, the trend of practice among bacteriologists in this country is to concentrate on the demonstration of *faecal coli* in water. Recently methods have been developed which enable a rapid enumeration of this organism, as apart from other organisms of the coliform group, to be made and it is anticipated that the quantitative estimation of *faecal coli* is likely in the future to play a dominant part in the routine control of water supplies. This does not mean to say that other types of coliform organisms can be neglected, because there is evidence to show that

some of these types survive longer in water than the *faecal coli* type, and hence may reveal by their presence more distant excretal contamination which, though not dangerous at the time of sampling, may nevertheless indicate that the water is subject to potentially dangerous pollution.

## B. TYPE OF EXAMINATION

Various tests are available for controlling the bacteriological purity of waters, such as the plate count at 22° C. and 37° C., the presumptive coliform count, the differential coliform test, and the tests for faecal streptococci and for *Clostridium welchii*. The particular test or combination of tests to be carried out in the routine supervision of waters must be left to the discretion of the bacteriologist but for his assistance some such scheme as the following is suggested.

### 1. Simple Examination

The presumptive \* coliform count, with or without differentiation of the individual types of organisms present.

For routine control work it is suggested that the presumptive coliform tests should be carried out at 37° C. and the results read after 24 or 48 hours. If differentiation of the coliform organisms is required, it should be set in hand at once.

With piped water or other supplies known to be fairly pure, it should suffice for the presumptive coliform test to inoculate one 50 ml. and five 10 ml. quantities of water into MacConkey broth. With non-piped wa-

ter, and with most surface or shallow well waters the quantities inoculated should usually comprise one 50 ml., five 10 ml., and five 1 ml.

### 2. Partial Examination

The presumptive coliform count, with or without differentiation together with plate counts at 22° C.\* and 37° C.†

This type of examination should not usually be required for routine control purposes, but may be demanded either when the simple method of examination reveals the presence of definite pollution of doubtful origin, or when coliform organisms are present belonging to non-faecal or irregular types, or when coliform organisms appear in very small numbers in a water from which they are usually absent, or when greater control is necessary after a heavy rainfall, or when a new supply is being taken into consideration, or for some other reason.

The plate counts add considerably to the time and cost of the examination and should be reserved for waters in which information additional to that yielded by the coliform test is required.

### 3. Full Examination

The presumptive coliform count, with or without differentiation, plate counts at 22° C. and 37° C., and tests for faecal streptococci and for *Clostridium welchii*.

A full bacteriological examination should seldom be necessary except when a new supply is being consid-

\* Presence of acid and gas in MacConkey broth after incubation for 24 or 48 hours at 37° C. constitutes a "presumptive positive."

\* Incubation period 3 days.

† Incubation period 2 days.

ered, or the results yielded by the coliform test are ambiguous or suspected of being misleading. If, for instance, coliform organisms of an unusual type appear in a large water supply, and it is doubtful whether they are or are not indicative of excretal pollution, a search for *faecal streptococci* and for *Cl. welchii* may be of considerable assistance in reaching a decision. Both of these organisms are of excretal origin, and their presence denotes contamination of the water.

The value of the *faecal streptococcal* test is estimated variously by different workers. Some regard it as of considerable value, whereas others are of the opinion that it adds little to the information yielded by the test for coliform bacilli. This discrepancy of opinion is largely due to the fact that very few studies of *faecal streptococci* in relation to water supplies have been carried out during recent years.

Many of the older observations were made under somewhat artificial conditions and at a time when the differentiation of faecal from non-faecal types of streptococci was less satisfactory than it is today. It is not definitely known whether *faecal streptococci* survive on the average for a shorter or a longer time in water than coliform bacilli. Observations by modern methods are required on this point. Until they are completed, the interpretation of the results of the faecal streptococcal test, particularly when negative, must remain doubtful.

The *Cl. welchii* test stands on much firmer ground. This organism forms spores, which survive for a consider-

ably longer time than coliform bacilli, and usually resist chlorination. Its presence in water indicates that faecal contamination has occurred, and the presence of *Cl. welchii*, in the absence of coliform bacilli, indicates that the contamination is not of very recent date. The chief value of the test would seem to lie in the detection of remote or intermittent pollution, especially in shallow well waters.

Supplies of this type are often distributed to only a small population and their frequent examination by the coliform test is not usually practicable. An occasional examination for coliform bacilli may yield misleading results, since these organisms may have died out since the last access of pollution. In such instances the demonstration of *Cl. welchii* will show that the water is subject to contamination, and will point to the desirability of taking precautions to safeguard the health of the consumer.

### C. INTERPRETATION OF RESULTS

The interpretation of the results of bacteriological examination in terms of hygienic quality of water demands not only careful consideration of all the relevant factors, but also a considerable experience.

#### 1. Agar Plate Count

Most of the bacteria developing at 20° to 22° C. (in 3 days) and not at 37° C. (in 2 days) are saprophytic types which are non-pathogenic to human beings. It may therefore be thought that the agar plate count at this temperature is immaterial so far as judgment of the hygienic quality of the water is concerned. To some



extent this is true, but it may be pointed out that the 22° C. agar plate count affords some indication of (1) the amount of food substance available for bacterial nutrition and (2) the amount of soil, dust, and other extraneous material that has gained access to the water. On general grounds the greater the number of organisms developing at this temperature, the larger is the amount of available organic matter present, and the less suitable is the water for human consumption.

The bacteria developing at 37° C. on the other hand, are mainly parasitic or potentially parasitic types, derived from soil, sewage, or excretal material, and must therefore be regarded with far greater severity than the harmless saprophytic organisms that develop at 22° C. While a high 22° C. count may often be met with in a water free from dangerous pollution, a high 37° C. count frequently indicates the access of sewage to the water.

The ratio of the count at 22° C. to that at 37° C. is chiefly of use in help-

ing to explain sudden fluctuations in the bacterial content of a water; the higher the ratio, the more probable is it that the bacteria are clean soil and water saprophytes and therefore of small significance. The ratio may depend somewhat on season and may tend to be low in summer and high in winter. In unpolluted water, the ratio of the 22° C. count to the 37° C. count is usually 10 or more to 1; in polluted waters it is usually below 10. But this rule is subject to so many exceptions that no weight can be attached to single observations. After chlorination of a supply the ratio has no significance; it is usually very low.

## 2. The Coliform Count

Useful as the 22° C. and 37° C. agar plate counts may be, they are far surpassed by the coliform test, which provides the most delicate index of excretal pollution. Reference may be made to Table 21 for a description of the different types of coliform bacilli that may be found in water. So far as the available evidence goes, *Bact. coli* type I appears

TABLE 21.—DIFFERENTIATION OF THE COLIFORM GROUP

	M.R.	V.P.	Growth in citrate	Indole	Gas in MacCon- key at 44°C.	Gelatin lique- faction 7 days	Probable Habitat
<i>Bact. coli</i> , type I, faecal..	+	—	—	+	+	—	Human and animal intestine.
<i>Bact. coli</i> , type II.....	+	—	—	—	—	—	Doubtful; probably not primarily intestinal.
Intermediate, type I....	+	—	+	—	—	—	Mainly soil.
Intermediate, type II....	+	—	+	+	—	—	Mainly soil.
<i>Bact. aerogenes</i> , type I...	—	+	+	—	—	—	Mainly vegetation.
<i>Bact. aerogenes</i> , type II...	—	+	+	+	—	—	Mainly vegetation.
<i>Bact. cloacae</i> .....	—	+	+	—	—	+	Mainly vegetation.
Irregular, type I.....	+	—	—	+	—	—	Human and animal intestine.
Irregular, type II.....	+	—	—	—	+	—	Doubtful.
Irregular, other types....			Reactions variable				Doubtful.

to be far and away the most frequent type of coliform organism present in the human and animal intestine. Apart from excretal contamination, it is rarely found outside the animal body. On the other hand, organisms of the intermediate-aerogenes-cloacae (I.A.C.) group appear to have their primary habitat in soil and on vegetation. Though they are often found in the intestinal canal, they are present as a rule in numbers far inferior to those of the coli I type. It is therefore convenient to refer to *Bact. coli* I as "faecal coli," and to the I.A.C. Group as "non-faecal." In doing so, however, it must be remembered that these terms are essentially relative and are governed by the frequency distribution of the two types.

The observations of various workers suggest that at ordinary temperatures members of the I.A.C. group tend on the whole to survive longer in water than *faecal coli*, and to be more resistant to chlorination, though other factors than temperature probably play a part in determining the relative length of survival of these organisms.

On the basis of these findings it may be expected that, if water is recently contaminated with excretal material, organisms of the *faecal coli* type will gain access to it in numbers considerably superior to those of the I.A.C. group. On the other hand, in water that has been contaminated a few weeks previously, or in water that has been insufficiently chlorinated, organisms of the I.A.C. group may exceed those of the *faecal coli* type. These assumptions are borne

out in practice by comparative counts made on different types of water.

It has, however, been found that in waters showing only a minor degree of pollution, as judged by the very small numbers of coliform organisms present, I.A.C. may exceed *faecal coli*. The reason for this is not altogether clear, but it seems probable that waters of this type are either being contaminated from cultivated soil, in which organisms of the I.A.C. group are usually abundant, or else are receiving excretal contamination at such infrequent intervals that organisms of the *faecal coli* type have largely perished by the time the water is examined. The former explanation is to some extent supported by the comparatively high proportion of I.A.C. in shallow wells and land springs as compared with upland surface waters.

Knowledge of the importance of the different coliform types is still very deficient, but the present position may be summarized, so far as it affects Great Britain, in terms such as the following:

(1) *Bact. coli* I is essentially an index of recent excretal pollution. The finding of this organism in water in more than minimal numbers can never be safely ignored.

(2) The presence of organisms of the I.A.C. group in water in the absence of *faecal coli* may be due either to (a) contamination of the water with soil; (b) contamination of the water with excretal material at a time sufficiently distant to allow *faecal coli* to die out; (c) contamination of the water with the excreta of a person who is discharging I.A.C. in almost pure culture; this must be relatively

uncommon; (d) inadequate treatment of an initially polluted water with chlorine, which has only succeeded in killing off the more susceptible *faecal coli*. Which of these explanations is correct may be determined only by inquiry into the source and history of the water.

(3) The finding of a high proportion of *faecal coli* among the total coliform organisms is indicative of heavy or recent excretal pollution. On the other hand, a result showing that the majority of the coliform organisms appear to belong to the I.A.C. or to irregular types may be regarded as indicative of a slight, infrequent, or remote excretal pollution; or perhaps, if no *faecal coli* are present at all, of simple contamination with soil that may or may not have been excretally polluted some time previously.

(4) In practice it is unwise to neglect completely the presence of organisms of the I.A.C. group. Even though no *faecal coli* can be found, their presence may indicate a minor degree of pollution which at any time might become serious. Their appearance in a water, particularly a deep well water, from which they are normally absent, sometimes heralds the advent of pollution, and enables steps to be taken in time to stop further pollution or, if this is impossible, to protect the consumer by suitable treatment of the water.

(5) In general terms, the presence of *faecal coli* denotes recent and possibly dangerous excretal contamination, which must be urgently attended to. The presence of I.A.C. in an untreated water suggests less recent contamination, which though not immedi-

ately dangerous is nevertheless sufficient to call for further steps toward obtaining greater purity of the supply. The presence of I.A.C. in a treated water suggests either inadequate treatment or the access of undesirable material to the water after treatment.

These general propositions demand a few qualifying remarks:

(a) Coliform bacilli may easily gain access to water during the collection of the sample. In many instances an apparently polluted water is found, when properly sampled, to be perfectly satisfactory.

(b) The finding of coliform bacilli in wells can sometimes be traced to pollution of the shaft or adit with soil washings, old sacking or other material serving as a suitable medium for the growth of organisms of the I.A.C. group. The underground water itself may be quite pure.

(c) Another source of coliform bacilli, particularly of the I.A.C. type, is provided by leather washers on pumps and taps, by jute packing, string, and similar substances.

(d) In the tropics, members of the I.A.C. group appear to be very much more abundant than in Great Britain, and their presence in water should probably not be regarded with the same degree of severity as that which we are recommending for this country.

(e) No satisfactory method is at present available for distinguishing between *faecal coli* of human and animal origin. In upland surface waters, particularly, contamination with *faecal coli* from sheep grazing in the neighborhood is by no means un-



common and reservoirs are often contaminated by birds. It is unwise, however, for the bacteriologist to neglect the presence of these organisms, since he cannot be certain that contamination of human origin has not also occurred. It is safer to regard all *faecal coli* as indicative of possibly dangerous contamination, though some allowance must be made, depending on the type of water and the experience of the bacteriologist, for the presence of these organisms when animal, but not human, contamination is known to be frequent or continual.

The interpretation of the *faecal streptococcal* and the *Cl. welchii* tests has already been sufficiently indicated.

#### D. SUGGESTED CLASSIFICATION OF WATERS

The bacterial flora of water is determined by so many factors which vary from one source of supply to another that it is impossible to lay down hard and fast standards for waters as a whole. As is pointed out in the introduction, our aim must rather be, on the basis of frequent examinations, to establish a standard for each individual water, departure from which may be at once viewed with suspicion. Nevertheless, experience has shown that it is justifiable to expect waters intended for public supply to come up to a certain standard of purity. The particular standard laid down must vary with the type of water—deep or surface water, filtered or unfiltered, chlorinated or unchlorinated. The standards must not be interpreted too rigidly; on the other hand, no serious departure from

them ought to be viewed with complacency. Experience alone will dictate to the bacteriologist whether, in any given supply, departure from the usual quality is significant or not, and he will always bear in mind that the less variation there is, the better.

A sudden rise in the bacterial population, particularly of the coliform group, after heavy rainfall will be regarded as indicative of potential danger, since experience has shown the sinister association of flood water and water-borne disease. The bacteriologist will also remember that in any quantitative standard far more attention must be attached to the coliform than to the plate count results.

Ideally, coliform bacilli should be absent from 100 ml. of water, but such an ideal standard would exclude many waters that may be and are consumed with impunity for indefinitely long periods. In setting out the following classification, a probable number of 2 coliform organisms per 100 ml. is being permitted in non-chlorinated piped supplies.

	Presumptive coliform count per 100 ml.
Class 1. Highly satisfactory .	less than 1
Class 2. Satisfactory . . . . .	1-2
Class 3. Suspicious . . . . .	3-10
Class 4. Unsatisfactory . . . .	greater than 10

Throughout the year 50 per cent of samples should fall into Class 1; 80 per cent should not fall below Class 2; and the remainder should not fall below Class 3.

In chlorinated piped supplies the water ought to come into Class 1.

#### 1. Remarks on Classification of Waters

In interpreting this classification it must be understood that, owing to

various sampling errors over which the bacteriologist has little or no control, a water may fall into a given class at one examination and into an adjacent class at another examination, even though no change has occurred meanwhile in the quality of the water. It is for this reason that only 80 per cent of samples of *non-chlorinated* waters throughout the year are expected to conform to a given class. The great majority of the samples ought to fall into Class 1 or 2. An occasional drop to Class 3 need not evoke any great alarm, but if the water is frequently assigned to Class 3, or drops to Class 4, it may be assumed that its purity is below standard.

If, in a *non-chlorinated* piped water supply, a fall occurs to Class 3, an immediate examination should be made to find out whether the coliform organisms are mainly of the faecal or non-faecal (I.A.C. and irregular) types. If they are found to be of the *faecal coli* type, then steps should be taken to insist on greater purity of supply. If they are of the non-faecal type, further steps toward greater purity of supply need not be taken at once, but analyses of the water should be made more frequently in case the appearance of non-faecal types heralds the advent of more serious pollution. With deep well, or other very pure waters, which normally fall into Class 1, a drop even to Class 2 should not be neglected, and an attempt should be made to detect any possible source of pollution.

If a *chlorinated* water known to have been originally polluted, shows the presence of presumptive coliform

organisms, an investigation into the efficiency and working of the treatment plant should be undertaken. *Efficient chlorination should yield a water free from coliform organisms in 100 ml.; that is, such a water should fall into Class 1 of the above grading.* Even making allowance for sampling and other errors, the appearance of these organisms in quantities of 100 ml., i.e., a fall to Class 2, should at once occasion misgivings as to the adequacy of the chlorination process.

## 2. *Water Taken on Consumer's Premises*

The classification laid down here for piped water supplies refers essentially to the quality of the water entering the distribution service and not to that of the water on the consumer's premises. Whatever the quality of the water entering the distribution service may be, some slight deterioration is liable to occur during its passage to the consumer. Organic matter may be sucked in to a variable extent through defective water mains, and further contamination may occur in service reservoirs and cisterns or from washers on the service taps.

Coliform organisms of the non-faecal type may gain limited access from these sources, but their appearance in any considerable numbers should be regarded with grave suspicion. The appearance of *faecal coli* among them suggests at once that undesirable material is gaining access to the water. It is difficult to lay down standards for water of this type, but if careful comparison between the water before and after distribution

shows that any considerable deterioration has occurred, particularly in relation to *Bact. coli* I, then measures should be taken to ascertain the source of the pollution.

### 3. *Small Rural Supplies*

As pointed out above, the classification suggested refers essentially to waters distributed to populations of more than minimal size. In rural districts, where a piped water supply may at present be economically unobtainable, and where reliance has to be placed largely on private supplies, it may be difficult to reach the standard of Class 3 or 4. In such instances everything possible should be done to prevent the access of pollution to the water.

By relatively simple measures, such as the removal of obvious sources of contamination from the catchment area, and by attention to the coping, brick lining, and covering of the well, it should usually be possible to reduce the coliform count for a shallow well water to a level between 10 and 25 per 100 ml. Persistent failure to do this, especially if the water frequently gives a presumptive coliform count of 50 or over per 100 ml., should lead as a rule to condemnation of the supply, though there are circumstances such as where the pollution appears to be mainly of non-human origin, when an exception may be made to this rule.

Once more insistence must be made on the importance of adequate *topographical examination*. No matter how satisfactory the bacteriologist's analysis may be, a water can never be regarded as safe which is exposed to known sources of pollution. Water

undertakers are advised to concentrate on the protection of their gathering grounds, and, when necessary, on the adequate treatment of the water supplied, and to use the bacteriologists's report mainly as a check on the success of their endeavors.

To adopt a *laissez-faire* policy and to do nothing until the bacteriologist's report shows that the pollution has reached a stage at which it can no longer be neglected is merely to court disaster. In rural areas, where frequent bacteriological examinations are impracticable, reliance must be placed almost exclusively on topographical examination.

### E. THE PRESUMPTIVE COLIFORM COUNT

In accordance with well established usage, the term "coliform" is here employed to include all Gram-negative non-sporing rods capable of fermenting lactose with the production of acid and gas, and of growing aerobically on agar media containing 0.5 per cent bile salt. Since the number of such organisms in water is usually small, the direct plate-counting method on a differential medium is not practicable. Often large quantities of water have to be inoculated to detect a single one, and for this reason recourse has to be made to the so-called "dilution method" of counting.

In this method, as used in general bacteriology, measured quantities of the bacterial suspension to be counted, or of one or more suitable dilutions, are inoculated into tubes of a liquid medium. These are incubated under appropriate conditions and observed for signs of growth. Provided a pro-



portion of the inoculated tubes remain sterile, it is assumed that every tube in which growth is manifest received an inoculum of at least one living bacillus. From the number of tubes showing growth in relation to the total number inoculated, it is possible by suitable probability tables to estimate the approximate number of living organisms in the original suspension.

As applied to the numerical estimation of coliform bacilli in water, the method has to take cognizance of the fact that these organisms are not present in pure culture. Attempts to overcome this difficulty generally take the form of choosing a selective medium containing lactose, in which members of the coliform group are the only organisms capable of growing and of fermenting the sugar with the production of acid and gas. Numerous media are available for this purpose. Without discussing their respective merits in detail, it may be pointed out that in this country MacConkey's lactose bile salt neutral red broth is widely used and has proved by experience to be admirably suited for this particular purpose.

The inclusion of bile salt serves to inhibit the growth of organisms of the aerobic spore-bearing group which, as American observers have found, are not infrequently responsible for false positive reactions in simple lactose broth. It is true that the bile salt has a slight inhibitory effect on the growth of the coliform organisms themselves, but this disadvantage is more than outweighed by the practically complete elimination of false positive reactions. With very few exceptions it may be taken that the

production of acid and gas in MacConkey medium inoculated with water indicates the presence of coliform bacilli. Since the conclusive proof of this can be brought only by further tests, a reaction of this sort is generally referred to as a "presumptive positive coliform reaction." The presumption is, in this country, however, very high. In other countries, where the water flora are different, false positive reactions in this medium may not infrequently be encountered.

### *1. The Sampling Error of the Coliform Count*

In the past it has been usual to report the results of the coliform test according to some such formula as the following:

Present in 100 ml.; absent from 10 ml.; or present in 10 ml.; absent from 1 ml.

Though this practice has something to recommend it, aiming as it does at only an approximate numerical estimate of the coliform bacilli present, it takes no account of the very large sampling error inherent in the dilution method of counting.

Various workers have devised formulae, based on the laws of probability, from which it is possible to calculate the number of organisms that are probably present when any given proportion of the tubes inoculated shows growth, or acid and gas production, or other characteristic change. McCrady (1918) and Hoskins (1934) have both published tables that are peculiarly suitable for use in water analysis. By means of

these tables it is possible to report the probable number of coliform bacilli per 100 ml. of water.

Unfortunately, as has already been pointed out, the dilution method of counting has a very large sampling error. The figures given by probable number tables, therefore, are subject to a wide margin of variation, and their use in practice is liable to be misleading unless the extent of this variation is constantly borne in mind.

How then are we to report the results of the coliform test? Fortunately what we really want to learn from the test is not the exact numbers of coliform bacilli present, but the maximum numbers that may be present. Supposing, for example, we allow 3 or 4 coliform bacilli in every 10 ml. of a piped water supply, and 15 every 100 ml. of a non-piped supply, we want to know how often the probable number of coliform bacilli obtained from the table is likely to under-estimate the real number. While it is true that the variations given above may occur occasionally, it must be remembered that these are extreme variations. In actual practice the great majority, somewhat about 80 per cent, of the probable figures given will fall between about one-half and double the real figure. If, for example, the real number of coliform bacilli per 100 ml. is 2, then a probable figure of between 1 and 4 bacilli per 100 ml. will usually be obtained. If the probable figure ex-

ceeds the real figure, the water may be unjustly condemned; but if the probable figure is less than the real figure, there is a danger that a contaminated water may be passed as satisfactory. It is this latter contingency which must be guarded against.

From Halvorson and Ziegler's work it would appear that the chance of obtaining a probable figure less than half the real figure is approximately 15 to 1 against. Consequently if the standard is based on the probability figures, we must be careful to select such a figure as will more or less guarantee that the real number of coliform bacilli present is not likely to exceed a dangerous limit.

If, for example, the probable figure given by the tables is 1 per 100 ml., then it is unlikely that the real figure will be greater than 2 per 100 ml. It is for this reason that the standards laid down may seem to be unnecessarily severe. In the interpretation of these standards, however, it must be remembered that the probable figure is as likely to over-estimate as to under-estimate the real figure. In practice, therefore, the occurrence of a probable figure exceeding the standard laid down is bound to occur fairly frequently even when the real number is within the standard. For practical purposes, provided the standard is not exceeded more than once in 5 or 10 examinations, no undue alarm need be experienced.

## APPENDIX II

### CHEMICALS AND REAGENTS

Analysts are advised to make use of the specifications for and grades of chemicals as listed by the American Chemical Society, whenever available, in carrying on the procedures outlined in this text. The materials for which specifications have been adopted are given in Sec. 1 of this Appendix.

As an aid in the simplification of this text, and to make for greater usability, the directions for the preparation of the ordinary laboratory reagents and indicators have been gathered together and placed in Sec. 2 of this Appendix.

#### 1. Reagent Grade Chemicals

The ACS specifications for chemical reagents have been published from time to time in *Industrial and Engineering Chemistry*. A book and supplement including all of the specifications and corrections may be obtained from the American Chemical Society, Washington, D. C., for a nominal sum. For those who wish to refer to the original publications, the number following each item in Table 22 refers to the bibliography and the issue of *Industrial and Engineering Chemistry*, in which the specification may be found. (Corrections in specifications, if any, appear in later references.)

TABLE 22.—REAGENT LIST—AMERICAN CHEMICAL SOCIETY SPECIFICATIONS

Acetic Anhydride .....	(2)	Aluminum and Potassium Sulfate ....	(2)
Acetone .....	(2)	Ammonium Acetate .....	(2)
Acid, Acetic, Glacial .....	(2)	Ammonium Carbonate .....	(2)
Acid, Benzoic .....	(11)	Ammonium Chloride .....	(2)
Acid, Boric .....	(11)	Ammonium Hydroxide .....	(1)
Acid, Citric .....	(5)	Ammonium Nitrate .....	(3)
Acid, Formic .....	(11)	Ammonium Oxalate .....	(1)
Acid, Hydriodic .....	(13)	Ammonium Persulfate .....	(2)
Acid, Hydrochloric .....	(1)	Ammonium Phosphate Dibasic (Diam-	
Acid, Hydrofluoric .....	(2)	monium Hydrogen Phosphate) ....	(3)
Acid, Molybdic Anhydride .....	(4)	Ammonium Sulfate .....	(2)
Acid, Molybdic, 85% .....	(4)	Ammonium Thiocyanate .....	(1)
Acid, Nitric .....	(1)	Amyl Alcohol (Isoamyl Alcohol) ....	(2)
Acid, Oxalic .....	(1)	Aniline .....	(11)
Acid, Perchloric .....	(2)	Arsenic Trioxide (A primary standard)	(4)
Acid, Perchloric, 60% .....	(13)	Barium Acetate .....	(9)
Acid, Phosphoric .....	(4)	Barium Carbonate .....	(3)
Acid, Sulfanilic .....	(11)	Barium Chloride .....	(1)
Acid, Sulfuric .....	(1)	Barium Hydroxide ( $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$ ) .	(3)
Acid, Sulfurous .....	(11)	Barium Nitrate .....	(4)
Acid, Tartaric .....	(5)	Benzene .....	(10)
Alcohol, Ethyl Absolute .....	(5)	Borax (Sodium Borate, $\text{Na}_2\text{B}_4\text{O}_7 \cdot$	
Alcohol, Ethyl .....	(5)	10 $\text{H}_2\text{O}$ ) .....	(12)



TABLE 22.—(Continued)

Bromine .....	(6)	Potassium Biphthalate .....	(11)
Cadmium Chloride ( $\text{CdCl}_2 \cdot 2\frac{1}{2}\text{H}_2\text{O}$ ) ..	(8)	Potassium Bisulfate Fused .....	(9)
Cadmium Sulfate .....	(9)	Potassium Bromate .....	(3)
Calcium Carbonate .....	(7)	Potassium Bromide .....	(3)
Calcium Carbonate, low in alkalies ...	(7)	Potassium Carbonate Anhydrous .....	(2)
Calcium Chloride Anhydrous .....	(5)	Potassium Chlorate .....	(3)
Calcium Chloride ( $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ) .....	(7)	Potassium Chloride .....	(2)
Carbon Disulfide .....	(4)	Potassium Chromate .....	(6)
Carbon Tetrachloride .....	(12)	Potassium Dichromate .....	(1)
Chloroform .....	(12)	Potassium Ferricyanide .....	(5)
Chromium and Potassium Sulfate ....	(12)	Potassium Ferrocyanide .....	(5)
Chromium Trioxide .....	(8)	Potassium Hydroxide .....	(1)
Cobalt Nitrate, not low in Nickel ....	(12)	Potassium Iodate .....	(3)
Cupric Acetate Normal		Potassium Iodide .....	(3)
( $\text{Cu}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot \text{H}_2\text{O}$ ) .....	(10)	Potassium Nitrate .....	(10)
Cupric Ammonium Chloride .....	(6)	Potassium Oxalate .....	(11)
Cupric Nitrate ( $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ ) ....	(10)	Potassium Permanganate .....	(2)
Cupric Oxide Powdered and Granular .	(6)	Potassium Phosphate, Monobasic (Po-	
Cupric Oxide, Wire Form .....	(7)	tassium Dihydrogen Phosphate) ...	(4)
Cupric Sulfate .....	(3)	Potassium Sulfate .....	(2)
Cuprous Chloride .....	(7)	Silver Nitrate .....	(1)
Dimethylglyoxime .....	(13)	Silver Sulfate .....	(6)
Ether, Absolute .....	(6)	Sodium Acetate .....	(3)
Ether .....	(6)	Sodium Bicarbonate .....	(2)
Ferric Ammonium Sulfate .....	(2)	Sodium Bismuthate .....	(3)
Ferric Chloride .....	(9)	Sodium Bisulfate Fused ( $\text{NaHSO}_4$ ) ..	(5)
Ferric Nitrate .....	(9)	Sodium Borate ( $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ ) ....	(9)
Ferrous Ammonium Sulfate (Not a		Sodium Carbonate Anhydrous .....	(2)
primary standard) .....	(2)	Sodium Chloride .....	(5)
Glycerol .....	(12)	Sodium Cyanide .....	(2)
Hydrogen Peroxide .....	(11)	Sodium Fluoride .....	(10)
8-Hydroxyquinoline .....	(12)	Sodium Hydroxide .....	(1)
Iodine .....	(1)	Sodium Metal .....	(7)
Lead Acetate .....	(7)	Sodium Nitrate .....	(4)
Lead Carbonate .....	(12)	Sodium Nitrite .....	(10)
Lead Chromate .....	(8)	Sodium Nitroferrocyanide .....	(8)
Lead Dioxide .....	(3)	Sodium Oxalate .....	(1)
Lead Nitrate .....	(8)	Sodium Peroxide .....	(4)
Lead Subacetate .....	(13)	Sodium Phosphate, Dibasic (Disodium	
Litmus Paper .....	(9)	Hydrogen Phosphate) .....	(13)
Magnesium Chloride ( $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ ) ..	(5)	Sodium Sulfate, Anhydrous .....	(2)
Magnesium Oxide .....	(8)	Sodium Sulfide .....	(4)
Magnesium Sulfate .....	(7)	Sodium Sulfite, Anhydrous .....	(11)
Manganese Sulfate Monohydrate ....	(13)	Sodium Thiosulfate .....	(2)
Mercuric Bromide .....	(11)	Sodium Tungstate .....	(12)
Mercuric Chloride .....	(8)	Stannous Chloride .....	(4)
Mercuric Oxide, Yellow .....	(13)	Toluene .....	(10)
Mercurous Chloride .....	(8)	Uranium Nitrate .....	(8)
Mercury .....	(12)	Xylene .....	(10)
Methanol .....	(5)	Zinc Chloride .....	(5)
Phenolphthalein .....	(10)	Zinc, low in arsenic, lead and iron ...	(13)
Phosphorous Pentoxide .....	(13)	Zinc Oxide .....	(12)
Potassium and Sodium Tartrate ....	(6)	Zinc Sulfate .....	(7)

TABLE 23.—U. S. BUREAU OF STANDARDS—STANDARDIZATION SAMPLES

Sample number	Name	Constituents determined or intended use
84	Acid potassium phthalate	Acidimetric value
39d	Benzoic acid	Acidimetric and calorimetric values
40c	Sodium oxalate	Oxidimetric value
83	Arsenic trioxide	do
38b	Naphthalene	Calorimetric value
17	Sucrose	Calorimetric and saccharimetric values
41	Dextrose	Reducing value

Certified samples of various items used in standardization may be obtained from the U. S. Bureau of Standards. Table 23 lists samples available.\*

#### BIBLIOGRAPHY

1. Committee on Analytical Reagents, *Ind. Eng. Chem.*, 17, 756 (1925).
2. Idem, *Ind. Eng. Chem.*, 18, 636, 759 (1926).
3. Idem, *Ind. Eng. Chem.*, 19, 645 (1927).
4. Idem, *Ind. Eng. Chem.*, 19, 1369 (1927).
5. Idem, *Ind. Eng. Chem.*, 20, 979 (1928).
6. Idem, *Ind. Eng. Chem., Anal. Ed.*, 1, 171 (1929).
7. Idem, *Ind. Eng. Chem., Anal. Ed.*, 2, 351 (1930).
8. Idem, *Ind. Eng. Chem., Anal. Ed.*, 3, 221 (1931).
9. Idem, *Ind. Eng. Chem., Anal. Ed.*, 4, 154 (1932).
10. Idem, *Ind. Eng. Chem., Anal. Ed.*, 4, 347 (1932).
11. Idem, *Ind. Eng. Chem., Anal. Ed.*, 5, 289 (1933).
12. Idem, *Ind. Eng. Chem., Anal. Ed.*, 12, 631 (1940).
13. Idem, *Ind. Eng. Chem., Anal. Ed.*, 16, 231 (1944).

#### 2. Common Laboratory Reagents

ACETIC ACID SOLUTION; 50 PER CENT.—Dilute 50 ml. of glacial acetic

acid with 50 ml. of boiled distilled water.

AMMONIUM HYDROXIDE SOLUTION; 1:1.—Mix 1 volume of concd.  $\text{NH}_4\text{OH}$  with 1 volume of distilled water.

AMMONIUM HYDROXIDE SOLUTION; 1:4.—Mix 1 volume of concd.  $\text{NH}_4\text{OH}$  with 4 volumes of distilled water.

AMMONIUM HYDROXIDE SOLUTION; 1:5.—Mix 1 volume of concd.  $\text{NH}_4\text{OH}$  with 5 volumes of distilled water.

AMMONIUM HYDROXIDE SOLUTION; 1:100.—Mix 1 volume of concd.  $\text{NH}_4\text{OH}$  with 100 volumes of distilled water.

ARSENOUS ACID SOLUTION; APPROX. 0.1 N.—Dissolve 5 g. of  $\text{As}_2\text{O}_3$  (reagent grad.) in 1 liter of distilled water.

BROM CRESOL GREEN INDICATOR SOLUTION.—Dissolve 0.1 g. of brom cresol green indicator in 14.3 ml. of 0.01 N NaOH. Dilute to 250 ml. with distilled water to obtain 0.04 per cent reagent.

ETHYL ALCOHOL SOLUTION; 1:1.—Dilute 1 volume of 95 per cent  $\text{C}_2\text{H}_5\text{OH}$  with 1 volume of distilled water.

\* Anon. Standard samples issued. *Bureau of Standards Supplement to Circular No. C398* (Feb. 18, 1935).

HYDROCHLORIC ACID SOLUTION; 1:1.  
—Mix 1 volume of concd. HCl with 1 volume of distilled water.

HYDROCHLORIC ACID SOLUTION; 1:8.  
—Mix 1 volume of concd. HCl with 8 volumes of distilled water.

HYDROCHLORIC ACID SOLUTION; 1:9.  
—Mix 1 volume of concd. HCl with 9 volumes of distilled water.

HYDROCHLORIC ACID SOLUTION;  
1:11.—Mix 1 volume of concd. HCl with 11 volumes of distilled water.

HYDROCHLORIC ACID SOLUTION;  
1:23.—Mix 1 volume of concd. HCl with 23 volumes of distilled water.

HYDROCHLORIC ACID SOLUTION;  
1:50.—Mix 1 volume of concd. HCl with 50 volumes of distilled water.

HYDROCHLORIC ACID SOLUTION; 6 N.  
—Dilute 560 ml. of concd. HCl to 1 liter with distilled water.

HYDROCHLORIC ACID SOLUTION; 5 N.  
—Dilute 465 ml. of concd. HCl to 1 liter with distilled water.

HYDROCHLORIC ACID SOLUTION; APPROX. 0:248 N.—Dilute 20 ml. of concd. HCl to 1 liter with distilled water.

HYDROCHLORIC ACID SOLUTION; 0.1 N.—Prepare a stock solution of approximately 1.0 N HCl by diluting 93 ml. of concd. HCl to 1 liter with distilled water. Standardize against pure anhydrous  $\text{Na}_2\text{CO}_3$ . Dilute the calculated volume of this 1 N HCl soln. to 1 liter to give a 0.1 N HCl soln. Standardize against pure anhydrous  $\text{Na}_2\text{CO}_3$ .

HYDROCHLORIC ACID SOLUTION; 0.02 N.—Dilute 200 ml. of 0.1 N HCl soln.

to 1 liter with distilled water. Standardize against standard 0.02 N  $\text{Na}_2\text{CO}_3$  soln.

IODINE SOLUTION; 0.025 N.—Dissolve approx. 2.0 g. of KI crystals in a small quantity of hot, boiled distilled water. Cool, add 3.173 g. of iodine and make up to 1 liter with distilled water. Standardize against 0.025 N  $\text{NaS}_2\text{O}_3$ .

METHYL ORANGE INDICATOR SOLUTION.—Dissolve 0.5 g. of methyl orange in 1 liter of distilled water. Keep the stock solution in the dark.

METHYL RED INDICATOR SOLUTION.—Dissolve 0.1 g. of methyl red with 7.4 ml. of 0.05 N NaOH solution. Dilute the solution to 100 ml. with distilled water.

NITRIC ACID SOLUTION; 1:1.—Mix 1 volume of concd.  $\text{HNO}_3$  with 1 volume of distilled water. Free the diluted acid from brown oxides of nitrogen, by aeration.

NITRIC ACID SOLUTION; 1:9.—Mix 1 volume of concd.  $\text{HNO}_3$  with 9 volumes of distilled water.

NITRIC ACID SOLUTION; 1:100.—Mix 1 volume of concd.  $\text{HNO}_3$  with 100 volumes of distilled water.

NITRIC ACID SOLUTION; 1 N.—Dilute 65 ml. of concd.  $\text{HNO}_3$  to 1 liter with distilled water. Free the solution of brown oxides of nitrogen, if any, by aeration. Standardize against pure anhydrous  $\text{Na}_2\text{CO}_3$ .

NITRIC ACID SOLUTION; 0.01 N.—Dilute 10 ml. of 1 N  $\text{HNO}_3$  soln. to 1 liter with distilled water. Standardize against 0.02 N  $\text{Na}_2\text{CO}_3$  soln.



**PHENOLPHTHALEIN INDICATOR SOLUTION.**—Prepare a 50 per cent solution of ethyl alcohol using freshly boiled and cooled distilled water. Dissolve 5 g. of phenolphthalein in 1 liter of the 50 per cent ethyl alcohol solution. Neutralize with 0.02 N NaOH.

**PHENOL RED INDICATOR SOLUTION.**—Dissolve 0.1 g. of phenol red in 5.7 ml. of 0.05 N NaOH and dilute to 100 ml. with distilled water.

**PHOSPHORIC ACID SOLUTION; 0.1 N.**—Prepare a stock solution of 1 N  $\text{H}_3\text{PO}_4$  by dissolving 23 ml. of concd. 85 per cent  $\text{H}_3\text{PO}_4$  in 1 liter of water. Standardize against pure anhydrous  $\text{Na}_2\text{CO}_3$ . Dilute 100 ml. of this 1 N soln. to 1 liter with distilled water and standardize.

**POTASSIUM HYDROXIDE SOLUTION; 12 N.**—Dissolve 672 g. of KOH in distilled water and make up to 1 liter.

**POTASSIUM HYDROXIDE SOLUTION; 0.1341 N.**—Prepare a stock solution of approx. 1 N KOH by weighing out approx. 70 g. of KOH sticks and dissolving off the surface coating in 200 ml. of distilled water. Pour off the supernatant and dissolve the washed sticks and make up to 1 liter with distilled water. Standardize against sulfuric acid. Dilute the calculated volume of this standard KOH to 1 liter with distilled water to obtain the 0.1341 N solution.

**POTASSIUM PERMANGANATE; APPROX. 0.2 N.**—Dissolve 6.3 g. of  $\text{KMnO}_4$  in distilled water and make up to 1 liter.

**POTASSIUM PERMANGANATE; 0.1 N.**—Dissolve 3.2 g. of  $\text{KMnO}_4$  in distilled water and make up to 1 liter. Standardize against sodium or ammo-

nium oxalate. Standardization is carried out hot in presence of dil.  $\text{H}_2\text{SO}_4$ .

**SODIUM CARBONATE; 0.02 N.**—Dissolve exactly 1.059 g. of dried pure anhydrous  $\text{Na}_2\text{CO}_3$  in 1 liter of freshly boiled and cooled distilled water.

**SODIUM HYDROXIDE SOLUTION; 2 N.**—Weigh approximately 100 g. of NaOH sticks. Place in approx. 300 ml. of distilled water until the surface coating dissolves. Pour off the supernatant liquor and dissolve the remaining NaOH (approx. 80 g.) in freshly boiled and cooled distilled water and dilute to 1 liter. Standardize with sulfuric acid using methyl orange as the indicator.

**SODIUM HYDROXIDE SOLUTION; 1.0 N, CARBONATE FREE.**—Prepare a saturated solution of NaOH and allow to stand stoppered (rubber) in a Pyrex flask. Sodium carbonate, being insoluble in saturated NaOH soln., settles to the bottom. Estimate the strength of the supernatant liquor by titration with sulfuric acid and dilute accordingly with freshly boiled and cooled distilled water. Keep the solution protected from the carbon dioxide of the air by guard tubes filled with soda lime or its equivalent. Standardize the solution against sulfuric acid using methyl orange as the indicator.

**SODIUM HYDROXIDE SOLUTION; 0.5 N, CARBONATE FREE.**—Prepare as directed for 1.0 N sodium hydroxide solution, carbonate free or dilute that solution according to its normality, if available, using freshly boiled and cooled distilled water. Protect against  $\text{CO}_2$  from the air.

SODIUM HYDROXIDE SOLUTION; APPROX. 0.25 N.—Dissolve 10 g. of NaOH in 100 ml. of distilled water.

SODIUM HYDROXIDE SOLUTION; 0.1 N.—Dilute 0.5 N or 1.0 N NaOH solutions, if available, with freshly boiled and cooled distilled water, or prepare as directed under those reagents.

SODIUM HYDROXIDE SOLUTION, 0.0231 N, STANDARD, CARBONATE FREE.—Prepare a saturated solution of sodium hydroxide and allow to stand stoppered (rubber) in a Pyrex flask. Sodium carbonate, being insoluble in saturated sodium hydroxide, settles to the bottom. Estimate the strength of the supernatant liquid by titration and dilute accordingly with freshly boiled and cooled distilled water. Keep the solution protected from the carbon dioxide of the air by guard tubes filled with soda lime or its equivalent. Standardize the solution against benzoic acid or acid potassium phthalate. (This solution may also be made by diluting 1.0 N or 0.5 N carbonate free NaOH if available.)

SODIUM HYDROXIDE SOLUTION; 0.02 N, STANDARD CARBONATE FREE.—Prepare as directed under *Sodium Hydroxide, 0.0231 N, carbonate free*, diluting to proper normality with freshly boiled distilled water.

SODIUM THIOSULFATE STANDARD SOLUTION; 0.025 N.—Prepare a stock solution of 0.1 N by dissolving exactly 24.84 g. of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  in 1 liter of freshly boiled and cooled distilled water. Add 1 per cent potassium furoate as a preservative (0.4 g. of NaOH or 5 ml. of chloroform per liter have also been used as preservatives).

Standardize the solution against potassium bi-iodate, potassium bichromate or potassium iodate. Dilute the calculated volume of this solution to 1 liter with freshly boiled and cooled distilled water to obtain the 0.025 N solution. Standardize.

STARCH INDICATOR SOLUTION.—To 5 g. of starch (potato, arrowroot or soluble) in a mortar, add a little cold water and grind to a thin paste. Pour into 1 liter of boiling distilled water, stir and allow to settle overnight. Use the clear supernatant liquor. The solution should be preserved with salicylic acid (1.25 g. per liter of starch solution). The starch may be preserved also with zinc chloride (4 g. per liter).

SULFURIC ACID SOLUTION; 1:1.—Mix 1 volume of concd.  $\text{H}_2\text{SO}_4$  with 1 volume of distilled water.

SULFURIC ACID SOLUTION; 1:3.—Mix 1 volume of concd.  $\text{H}_2\text{SO}_4$  with 3 volumes of distilled water.

SULFURIC ACID SOLUTION; 1:6.—Mix 1 part of concd.  $\text{H}_2\text{SO}_4$  with 6 parts of distilled water.

SULFURIC ACID SOLUTION; 1:8.—Mix 1 part of concd.  $\text{H}_2\text{SO}_4$  with 8 parts of distilled water.

SULFURIC ACID SOLUTION; 1:35.—Mix 1 volume of concd.  $\text{H}_2\text{SO}_4$  with 35 volumes of distilled water.

SULFURIC ACID SOLUTION; 2 N.—Dilute 60 ml. of concd.  $\text{H}_2\text{SO}_4$  to 1 liter with distilled water. Standardize against pure anhydrous  $\text{Na}_2\text{CO}_3$ .

SULFURIC ACID SOLUTION; 1 N.—Dilute 30 ml. of concd.  $\text{H}_2\text{SO}_4$  to 1 liter with distilled water, or dilute 500 ml.

of 2 N  $\text{H}_2\text{SO}_4$  soln. to 1 liter. Standardize against pure anhydrous  $\text{Na}_2\text{CO}_3$

SULFURIC ACID SOLUTION; 0.1 N.—Dilute 100 ml. of 1 N  $\text{H}_2\text{SO}_4$  soln. or 50 ml. of 2 N  $\text{H}_2\text{SO}_4$  soln. to 1 liter with distilled water. Standardize against pure anhydrous  $\text{Na}_2\text{CO}_3$ .

SULFURIC ACID; 0.02 N.—Prepare a stock solution of approx. 0.2 N by diluting 6 ml. of concd.  $\text{H}_2\text{SO}_4$  to 1 liter with distilled water. Standardize against pure anhydrous  $\text{Na}_2\text{CO}_3$ . Dilute an exact quantity of this stock solution (depending on its normality) to 1 liter with distilled water.



## INDEX

Acetic Acid Solution—50% .....	248
Acidity	
Sewage .....	146
Sludge .....	158
Water .....	31
Mineral .....	31
Total .....	31
Volumetric .....	31
Acids	
(See individual acid)	
Activated Sludge	
Activity of .....	219
Density index (S.D.I.) .....	158
Liquor, dissolved oxygen .....	134, 136
Oxygen demand of .....	219
Settleability .....	157
Suspended solids .....	157
Volume index (S.V.I.) .....	158
Activity of Activated Sludge .....	219
Adjustment of Reaction (Culture Media) .....	184
Agar .....	184
Bile salt .....	228
Brilliant green lactose bile .....	228
British practice .....	233
Endo .....	187
Eosin methylene-blue .....	187, 228
Ferrocyanide citrate .....	228
MacConkey .....	228
Methylene-blue erythrosine brom-cresol-purple .....	228
Nutrient .....	186
Selective for coliform group .....	227
Tryptone glucose extract .....	186
Violet red .....	228
Albuminoid Nitrogen in Water .....	68
Aliquot Portion (Microscopic Exam.) .....	170
Alkaline Hypochlorite Method for D.O. ....	132, 136
Alkaline-Iodide Reagent .....	108, 127
Sodium azide .....	130
Alkalinity	
Sewage .....	147
Sludge .....	158
Water .....	31
Bicarbonate from .....	9, 37, 38
Carbonate from .....	9, 38
Hydroxide from .....	9, 42
Methyl orange .....	9, 32
Phenolphthalein .....	9, 32
Relation of methyl orange to phenolphthalein .....	9

Total .....	9, 31
Volumetric .....	31
Alpha-naphthylamine Reagent for Nitrites .....	72
Alsterberg Method for D.O. ....	129, 135
Alum Flocculation Method for D.O. ....	134, 138
Aluminon Reagent for Aluminum .....	51
Aluminum .....	50
Aluminon reagent for .....	51
Aurin tricarboxylic acid reagent for .....	51
Colorimetric method for .....	50
Free distilled water .....	50
Gravimetric method for .....	50
Iron and chromium interference with .....	50
Standards .....	51
Amer. Chem. Soc. Reagent Specifications .....	246
Amino-naphthol-sulfonic Acid Reagent for Orthophosphate .....	80
Ammonia Nitrogen .....	
Free water .....	65
Nessler reagent for .....	65
Sewage .....	115
Direct nesslerization method .....	116
Distillation method .....	117
Standards .....	67
Standard solution of .....	66
Water .....	64
Direct nesslerization method .....	67
Distillation method .....	65
Ammonium Hydroxide Solution .....	
1:1 .....	248
1:4 .....	248
1:5 .....	248
1:100 .....	248
Ammonium Molybdate Reagent for Orthophosphate .....	79
Apparatus .....	
(See "Laboratory Apparatus") .....	
(See the individual determination desired) .....	
Activity of activated sludge .....	220
Bacteriological .....	183
Bottom mud sampling .....	175
Dissolved oxygen sampling .....	106, 107, 125
Electronic titration .....	88
Evolution of CO <sub>2</sub> .....	33
Fluoride distillation .....	28
Methane sampling .....	111
Microscopic .....	164, 165
Organic carbon .....	225
Application of Methods for Residual Chlorine .....	92
Arsenic—Gutzeit Method .....	46
Arsenous Acid Solution—0.1N, approx. ....	248
Asbestos Cream .....	145
Ash in Solids .....	
Sewage .....	145
Water .....	20

Atomic Weights .....	1
Aurin Tricarboxylic Acid .....	51
Available Chlorine	
Combined .....	102
Free .....	102

### *Bacteriological Examination*

#### Agar

British practice .....	233
Endo .....	187
Eosin methylene-blue .....	187
Nutrient .....	186
Selective for coliform group .....	227
Tryptone glucose extract .....	186

Apparatus .....	183
Dilution bottles .....	183
Fermentation tubes .....	183
Petri dishes .....	183
Pipettes .....	183
Sample bottles .....	183

British practice .....	233
------------------------	-----

#### Broths

Brilliant green lactose bile .....	188
Formate ricinoleate .....	188
Lactose .....	186
Lauryl sulfate tryptose .....	188
Selective for primary or parallel planting .....	226

#### Coliform group

Completed test .....	194, 196, 199, 201
Confirmed test .....	194, 195, 199, 200
Definitions .....	193
Differentiation .....	194, 227
Estimating density .....	202
Gram stain .....	198
Interpretation of results .....	207
Presumptive test .....	193, 194, 199
Selection of tests for .....	199
Standard tests .....	193
Swimming pool .....	207

Collection of samples .....	190
-----------------------------	-----

Counting .....	192
----------------	-----

#### Culture media

Adjustment of reaction .....	184
Brilliant green lactose bile broth .....	188
Broths for primary or parallel planting .....	226
Concentration of ingredients .....	188
Dehydrated .....	188
Endo .....	187
Eosin methylene-blue agar .....	187
Formate ricinoleate broth .....	188
Lactose broth .....	186
Lauryl sulfate tryptose broth .....	188
Nutrient agar .....	186



*Bacteriological Examination (cont.)*

Nutrient gelatin .....	186
Permissible variations .....	188
Preparation of .....	184
Selective agar for coliform differentiation .....	227
Storage of .....	189
Tryptone glucose extract agar .....	186
Culture purification, coliform group .....	228
Differentiation, coliform group .....	194, 226, 227, 228
Dilutions .....	191
Eijkman test (coliform group) .....	231
Incubation .....	192
Indole test (coliform group) .....	230
Materials	
Agar .....	184
Chemicals .....	184
Dyes .....	184
Gelatin .....	184
Meat extract .....	184
Peptone .....	184
Sugars .....	184
Water .....	184
Methyl red test (coliform group) .....	230
Plating .....	191
Reaction classification (coliform group) .....	239
Samples	
Collection .....	190
Storage .....	191
Transportation .....	191
Slow lactose fermenters (coliform group) .....	230
Sodium citrate test (coliform group) .....	231
Swimming pool control .....	207
Bacterial count .....	208
Coliform group .....	208
Preparation of sample bottle .....	207
Sample collection .....	208
Voges-Proskauer test (coliform group) .....	231
Barium Chloride Method	
For hydroxide .....	41
For metaphosphate .....	82
Benzidine Hydrochloride Reagent .....	84
Benzidine Method for Sulfate .....	84
Bicarbonate .....	37
Expression of results .....	9
From alkalinity .....	9, 37
From alkalinity and pH .....	38
From total carbon dioxide .....	37
Bile Salt Agar .....	228
Biochemical Oxygen Demand .....	139
Calculation of .....	141-142
Dilution method for	
Sewage .....	139
Sludges and muds .....	143

Procedure for	
Chlorinated sewage .....	142
Unchlorinated sewage .....	140
Seeding .....	140
Standard dilution water .....	140
Bipyridine Method for Iron .....	51
Boiler Feed Water Determinations .....	10
Boiler Water Determinations .....	10
Boron .....	87
Electrometric titration method .....	88
Bottles (Water Sample) .....	4
Bottom Sediments (Microscopic Exam.) .....	177
Brilliant Green Lactose Bile	
Agar .....	228
Broth .....	188
British Practice—Bacteriological Examination .....	233
Brom Cresol Green Indicator Solution .....	248
Broths, Culture	
Brilliant green lactose bile .....	188
British practice .....	233
Crystal violet .....	226
EC medium .....	226
Eijkman .....	226
Formate ricinoleate .....	188
Fuchsin lactose .....	226
Lactose .....	186
Lauryl sulfate tryptose .....	188
Methylene-blue erythrosine bromocresol-purple .....	226
Selective for primary or parallel planting .....	226
Buffer	
pH standards .....	211
Solutions for chlorine standards .....	95
Calcium	
Gravimetric method for .....	59
Hardness .....	23, 24, 25
Volumetric method for .....	60
Calculations	
Bicarbonate .....	9, 37
From alkalinity .....	9, 37
From alkalinity and pH .....	38
From carbon dioxide .....	37
Biochemical oxygen demand .....	141-142
Carbonate	
From alkalinity .....	9, 39
From alkalinity and pH .....	39
From carbon dioxide .....	38
Dissolved oxygen .....	138
Free carbon dioxide .....	34
Hydroxide	
From alkalinity .....	9, 42
From pH .....	42
Microscopic examination .....	174
Residue in water .....	20
Total hardness .....	23

## Calibration

Conductivity cell .....	21
Microscope .....	169

Candle Turbidimeter .....	11
---------------------------	----

Carbamate Method for Copper .....	47
-----------------------------------	----

## Carbonate

Barium chloride method for .....	39
Expression of results .....	8
From alkalinity .....	9, 39
From alkalinity and pH .....	39
From carbon dioxide .....	38
Normal .....	8

## Carbon Dioxide

Bicarbonate from .....	37
By evolution .....	32, 33
Carbonate from .....	38
Free or uncombined .....	34
By calculation .....	34
By titration .....	34
Total .....	32

Carbon, Organic, in Sewage .....	224
----------------------------------	-----

Cell Constant, Determination of Conductivity .....	21
--	----

Centrifuge for Microscopic Examination .....	163
--	-----

Characteristics of Odors .....	19
--------------------------------	----

## Chemical Results

Classification of .....	9
Expression of .....	5
Hypothetical combinations .....	7
Units .....	5

Chemicals and Reagents .....	246
------------------------------	-----

Common laboratory .....	248
-------------------------	-----

Standardization samples .....	248
-------------------------------	-----

Chloride .....	73
----------------	----

Mohr method .....	73
-------------------	----

Sewage .....	147
--------------	-----

Volhard method .....	73
----------------------	----

Chlorinated Sewage, B.O.D. of .....	142
-------------------------------------	-----

## Chlorine

Demand .....	103, 150
--------------	----------

Demand free water .....	92, 94
-------------------------	--------

## Residual

Sewage .....	142
--------------	-----

Water .....	92
-------------	----

Solution for standards .....	95
------------------------------	----

Standards .....	94-97
-----------------	-------

Water (reagent) .....	151
-----------------------	-----

## Chlorine Demand

Sewage .....	150
--------------	-----

Chlorine water for .....	151
--------------------------	-----

Definition .....	151
------------------	-----

Water .....	103
-------------	-----

Field method .....	104
--------------------	-----

Laboratory method .....	103
-------------------------	-----



Chromium .....	55
Colorimetric method .....	55
Diphenyl carbazide reagent .....	55
Hexavalent .....	56
Interference with aluminum detr. ....	50
Total .....	56
Clark Degrees of Hardness .....	6
Classification of Chemical Results .....	9
Coagulated Grease .....	223
Cobaltous Chloride (Color detr.) .....	14
Cold Odor .....	17
Coliform Group Bacteria .....	193
Agar for differentiating .....	227
Completed test .....	194, 196, 199, 201
Confirmed test .....	194, 195, 199, 200
Definitions .....	193
Estimating density .....	202
Drinking water .....	202
General considerations .....	202
Indicated number .....	205
Most probable number .....	203, 204, 205
Non-drinking water .....	203
Precision of tests .....	206
U. S. P. H. S. standards .....	202
Gram stain .....	198
Interpretation of results .....	207
Presumptive test .....	193, 194, 199
Selection of tests for .....	199
Standard tests .....	193
Swimming pool .....	207
Collection of Samples .....	
Bacteriological .....	196
Microscopic examination .....	
Sewage .....	178
Water .....	166
Sewage .....	113
Swimming pool .....	208
Water .....	1
Color .....	
Comparison with glass discs .....	15
Comparison with platinum-cobalt stds. ....	14
Platinum-cobalt standards .....	14
Procedure for determining .....	15
Recording results .....	14
Sewage .....	115
Colorimetric Apparatus .....	5
Colorimetric Determinations .....	
Aluminum .....	50
Ammonia .....	64
Arsenic .....	46
Chlorine, residual .....	92
Chromium .....	55
Copper .....	47

Cyanide .....	90
Iodide .....	74
Iron .....	51, 52, 53
Lead .....	49
Magnesium .....	61
Manganese .....	56, 57
Nitrogen .....	64-71
Orthophosphate .....	79
pH .....	30, 224
Potassium .....	64
Silica .....	44
Sulfides .....	87, 154
Combined Available Chlorine .....	102
Compensation for Interferences in Ortho-tolidine Test .....	98
Completed Test (Bact. Exam.) .....	194, 196, 199, 201
Concentration of Acid in Ortho-tolidine Reagent .....	92
Concentration of Samples	
Microscopic examination	
Water .....	163
Condensate	
Residue in .....	22
Silica in .....	45
Condensed Steam, Residue in .....	22
Confirmed Test (Bact. Exam.) .....	194, 195, 199, 200
Contact Time (Residual Chlorine Test) .....	92
Containers (Microscopic Exam.) .....	177
Conversion Table	
Data and factors for analytical results .....	6, 7
Hardness .....	6
Cooling Coil for Sampling Boiler Water .....	2, 3
Copper .....	47
Colorimetric method for .....	47
In iron-containing sample .....	48
In iron-free sample .....	48
Corrosive Constituents in Water .....	9
Counting Bacteria .....	192
Swimming pool .....	207
Counting Cell (Microscopic Exam.) .....	165
Crystal Violet Broth .....	226
Cubic Standard Units (Microscopic Exam.) .....	174
Culture Media .....	184
Adjustment of reaction .....	184
Bile salt agar .....	228
Brilliant green lactose bile agar .....	228
Brilliant green lactose bile broth .....	188
British practice .....	233
Clarification .....	185
Concentration of ingredients .....	188
Dehydrated .....	188
EC medium broth .....	226
Eijkman broth .....	226
Endo medium .....	187
Eosin methylene-blue agar .....	187

Ferrocyanide citrate agar .....	228
Formate ricinoleate broth .....	188
Fuchsin lactose broth .....	226
Lactose broth .....	188
Lauryl sulfate tryptose broth .....	188
MacConkey agar .....	228
Methylene-blue erythrosine bromeresol-purple agar .....	228
Methylene-blue erythrosine bromeresol-purple broth .....	226
Nutrient agar .....	186
Nutrient gelatin .....	186
Permissible variations .....	188
Preparation .....	184
Selective agar for coliform differentiation .....	227
Selective broth for primary or parallel planting .....	226
Sterilization .....	185
Storage of .....	189
Tryptone glucose extract agar .....	186
Violet red agar .....	228
Culture Purification .....	228
Cyanide, Colorimetric Method for .....	90
Definitions	
Chlorine demand .....	151
Coliform bacteria .....	193
Microscopic examinations .....	162, 177
Turbidity .....	10
Degrees of Hardness	
Clark .....	6
French .....	6
German .....	6
Dehydrated Media .....	188
Density Coliform Bacteria .....	202
Determinations	
Boiler feed water .....	10
Boiler water .....	10
Railroad supply water .....	10
Devices	
Collecting samples for microscopic exam. ....	162
Concentrating samples for microscopic exam. ....	163
Diammonium Hydrogen Phosphate Reagent for Magnesium .....	60
Difference Method	
For metaphosphate .....	83
For pyrophosphate .....	82
Differentiation (Bact. Exam.) .....	194
Coliform group .....	228
Agar for .....	227
Culture purification .....	228
Eijkman test .....	231
Indole test .....	230
Methyl red test .....	230
Reaction classification .....	239
Slow lactose fermenters .....	230
Sodium citrate test .....	231
Voges-Proskauer test .....	231



Dilution	
Bottles .....	183
Method for B.O.D.	
Sewage .....	139
Sludges and muds .....	143
Water for B.O.D. ....	140
Dilutions (Bact. Exam.) .....	191
Diphenyl Carbazide Reagent for Chromium .....	55
Dipyridil Method for Iron .....	51
Direct Nesslerization Method for Ammonia .....	67
Discussion of Residual Chlorine .....	92
Dissolved Oxygen	
Sewage. ....	124
Activated sludge .....	134, 136
Alkaline hypochlorite method .....	132, 136
Alsterberg method .....	129, 135
Alum flocculation method .....	134, 138
Calculation of results .....	138
Outline of procedure for .....	135
Rideal-Stewart modification .....	130
Sampler for .....	125
Samples for .....	124
Selection of method for .....	126
Sodium azide method .....	129, 135
Standardization of thiosulfate for .....	126
Winkler method .....	127, 135
Water .....	106
Sampling flask for .....	106, 107, 108
Dissolved Residue	
Sewage .....	146
Water .....	146
Dissolved Sulfides	
Sewage. ....	153, 155
Water. ....	87
Distillation	
Assembly for fluoride .....	78
Method for ammonia .....	65
Method for fluoride .....	76
Distilled Water .....	4
Dithiozone Method for Lead .....	214
Dredges (Microscopic Examination) .....	177
Drop Dilution Method for Residual Chlorine .....	102
Duboseq Colorimeter .....	5
(See individual colorimetric determinations)	
Dyes (Bact. Exam.) .....	184
EC Medium Broth .....	226
Eijkman Test (Bact. Exam.) .....	231
Broth .....	226
Electrolytic Conductivity for Residue	
Calibration .....	21
Cell constant .....	21

Electrometric Determination	
Boron, titration method	88
pH	28
Endo Medium	187
Enumeration of Organisms (Microscopic Exam.)	
Sewage	179
Water	170
Eosin Methylene-Blue Agar	187
Equivalents per Million to Parts per Million, Table for	7
Estimating Coliform Density	202
Drinking water	202
General considerations	202
Indicated number	205
Most probable number	205
Non-drinking water	203
Precision of test	203
U.S.P.H.S. standards	202
Ethyl Alcohol Solution—1:1	248
Evaporation and Extraction of Oil	42
Evaporation for Residue Determination	20
Evolution Method	
Carbon dioxide	32, 33
Sulfides	87
Examination of	
Industrial wastes	113
Muds	156, 177
Polluted waters	113
Sewage	113
Sewage effluents	113
Sludges	156, 177
Water	1
Bacteriological	183
Chemical	20-112
Introduction to	1
Microscopic	162
Physical	10-19
Expression of Results	
Alkalinity	9
Bacteriological	192, 202
Chemical (see chemical results)	5
Microscopic	172-180
Sewage	114
Extraction of Oil	
Evaporation and	42
Wet	43
Factors to Convert e.p.m. to p.p.m. and Reverse	7
False End Point	26
Fats (see grease)	
Fermentation Tubes	183
Ferric Iron	51
Colorimetric	
Bipyridine method	51

Phenanthroline method .....	52
Thiocyanate method .....	53
Gravimetric .....	51
Standards .....	54
Ferrocyanide Citrate Agar .....	228
Ferrous Iron	
Colorimetric	
Bipyridine method .....	51
Phenanthroline method .....	52
Standards .....	54
Field Count (Microscopic Exam.) .....	170
Field Method	
Chlorine demand .....	104
Residual chlorine .....	151
Fixed Solids (Residue)	
Sewage .....	145
Water .....	20
Flash Test for Residual Chlorine .....	92, 100
Fluoride	
Distillation assembly .....	78
Sanchis' method for .....	76
Scott modification method for .....	76
With distillation .....	77
Without distillation .....	76
Zirconium alizarin reagent for .....	76
Foerst Centrifuge .....	163
Folin's Ammonia Permutit .....	65
Formate Ricinoleate Broth .....	188
Forms (Microscopic Exam.) .....	172, 173
Free Available Chlorine .....	102
Free Carbon Dioxide	
By calculation .....	34
By titration .....	34
French Degrees of Hardness .....	6
Fuchsin Lactose Broth .....	226
Fullers' Earth for Turbidity Standards .....	12
Funnel and Centrifuge (Microscopic Exam.) .....	164
Gelatin, Nutrient .....	186
General Chemicals (Bact. Exam.) .....	184
German Degrees of Hardness .....	6
Glass Discs for Color .....	15
Glass Electrode .....	28, 29
Glassware, Volumetric .....	5
Gooch Crucibles, Preparation of .....	21, 145
Gram Stain .....	198
Gravimetric Determinations	
Aluminum .....	50
Calcium .....	59
Iron .....	51
Magnesium .....	60
Orthophosphate .....	79
Potassium .....	66



Silica .....	44
Sodium .....	62
Sulfate .....	83
Grease .....	
Sewage .....	155
Sludge .....	158
Coagulated grease .....	223
Wet extraction .....	221
Gutzeit Method for Arsenic .....	46
Hardness .....	23
Calcium .....	23, 24, 25
Calculation .....	23
Carbonate .....	23
Conversion table .....	6
Degrees (Clark, French, German) .....	6
False end points .....	26
Lather factor .....	25
Low (zeolite effluent) .....	25
Magnesium .....	23, 24, 25
Non-carbonate .....	27
Palmitate .....	23
Soap .....	25
Soda reagent .....	27
Total .....	23, 25, 27
Hexavalent Chromium .....	56
Hot Odor .....	17
Hydrochloric Acid Method for Silica .....	44
Hydrochloric Acid Solution .....	
1:1 .....	249
1:8 .....	249
1:9 .....	249
1:11 .....	249
1:23 .....	249
1:50 .....	249
6N .....	249
5N .....	249
0.248N .....	249
0.1N .....	249
0.02N .....	249
Hydrogen Ion Concentration .....	28
(See pH)	
Hydrogen Sulfide .....	
Sewage .....	153, 155
Water .....	110
Free .....	111
Sampling .....	110
Uncombined .....	111
Hydroxide .....	40
Barium chloride method for .....	41
Expression of results .....	8
From alkalinity .....	9, 42
From pH .....	42

Strontium chloride method for .....	40
Hypothetical Combinations .....	7
Ignition, Loss on .....	20, 145
Immediate Chlorine Demand .....	151
Incrustants .....	9
Incubation (Bact. Exam.) .....	192
Indicated Number (Bact. Exam.) .....	205
Indicators	
pH .....	30, 213
(See individual determinations)	
Indole Test (Bact. Exam.) .....	230
Interference	
Colors in OTA test .....	102
Iron in aluminum determination .....	50
Iron in copper determination .....	48
Substances in ortho-tolidine test .....	93, 98
Interfering Colors in OTA Test .....	102
Interpretation of Results	
Bacteriological examination .....	207
Microscopic examination .....	172
Interval Before Analysis, Time .....	1
Introduction	
Examination of water .....	1
Iodide .....	74
Modified McClendon method for .....	75
Standards .....	76
Iodine Solution—0.025N .....	249
Iodometric Method for Residual Chlorine .....	93, 98
Iron .....	51
Colorimetric method for	
Bipyridine (dipyridil) .....	51
Phenanthroline .....	52
Thiocyanate .....	53
Ferrie .....	51
Ferrous .....	51, 52
Free distilled water .....	52
Gravimetric method for .....	51
Interference with aluminum .....	50
Interference with copper .....	48
Standards .....	54, 55
Standard solution .....	52, 53
Total .....	52, 53
Kjeldahl Method for Organic Nitrogen .....	68
In sewage .....	118
Laboratory Apparatus .....	4
(See also the individual determinations)	
Bottles .....	4
Colorimetric .....	5
Distilled water .....	4
Microscopic .....	162, 177

Nessler tubes .....	4
Photometric .....	5
Reagents .....	5
Specialized .....	5
Volumetric .....	5
Laboratory Method	
Chlorine demand .....	103
Residual chlorine (spot test) .....	151
Laboratory Reagents, Common .....	248
See Reagents, common laboratory	
Lactose Broth .....	186
Lather Factor .....	25
Lauryl Sulfate Tryptose Broth .....	188
Laux Flash Test .....	100
Lead .....	49
Colorimetric method for .....	49
Non-standard method .....	214
Sulfide, determination as .....	49
Light Sources for Ortho-tolidine Test .....	94
Lignin and Tannin .....	91
Limitations in Sulfide Determination .....	155
Loss on Ignition .....	20, 145
MacConkey Agar .....	228
McClendon Method for Iodides .....	74
Magnesium .....	60
Colorimetric method for .....	61
Diammonium hydrogen phosphate reagent for .....	60
Free distilled water .....	61
Gravimetric method for .....	60
Hardness .....	23, 24, 25
Standards .....	61
Standard solutions of .....	61
Titan yellow reagent for .....	61
Manganese .....	56
Colorimetric methods for .....	56, 57
Periodate .....	56
Persulfate .....	57
Interference with aluminum determination .....	50
Sodium paraperiodate reagent for .....	56
Standards .....	57
Standard solution of .....	56
Manganous Chloride Method for Pyrophosphate .....	81
Materials for Bacteriological Examination .....	184
Measurement of Turbidity .....	12
Meat Extract (Bact. Exam.) .....	184
Media (See Culture Media) .....	184
Metaphosphate	
Barium chloride separation method for .....	82
Difference method for .....	83
Methane .....	111
Methylene-blue .....	144



Methylene-blue Erythrosine Bromcresol-Purple	
Agar .....	228
Broth .....	226
Methyl Orange	
Alkanlinity .....	9, 32
Indicator solution .....	249
Methyl Red	
Indicator solution .....	249
Test (Bact. Exam.) .....	230
Microscopes .....	164
<i>Microscopic Examination</i>	
Apparatus	
Sewage sludge, sediments .....	177
Alternative .....	177
Qualitative samples .....	177
Quantitative samples .....	177
Containers .....	177
Dredges .....	177
Sieves .....	177
Water	
Devices for collecting samples .....	162
Devices for concentrating samples .....	163
Alternative method .....	164
Centrifuge method .....	163
Sedgwick-Rafter method .....	163
Examination	
Counting cell .....	165
Microscopes .....	164
Ocular micrometer .....	165
Stage micrometer .....	166
Bottom sediments .....	177
Collection of samples	
Sewage sludge, sediments .....	178
Water .....	166
Concentration of samples	
Sewage sludge, sediments .....	178
Water .....	167
Definitions and scope	
Bottom sediments and sludge .....	177
Sewage sludge .....	177
Water .....	162
Examination	
Sewage sludge, sediment .....	179
Enumeration of organisms .....	179
Water samples .....	169
Aliquot portion .....	170
Calibration of microscope .....	169
Field count .....	170
Strip count .....	171
Survey count .....	173
Reporting results	
Forms .....	181-182
Sewage, sludge, sediments .....	180

*Microscopic Examination (cont.)*

Water .....	173
Calculation .....	174
Cubic standard units .....	174
Number of organisms .....	176
Forms .....	172, 173
Observations .....	173
Sample collection	
Sewage sludge .....	178
Water .....	166
Modified McClendon Method for Iodides .....	74
Modified Scott Standards for Residual Chlorine .....	92, 97
Mohr Method for Chloride .....	73
Moisture in Sludge .....	159
Molybdate Method for Silica .....	44
For testing condensate .....	45
Sodium sulfite reduction of .....	46
Most Probable Number (Bact. Exam.) .....	203
Nephelometric Determinations	
Zinc .....	58
Nesslerization Method for Ammonia .....	65
Nessler's Reagent for Ammonia .....	65
Nessler Tubes .....	4
Nitrate Nitrogen	
Phenoldisulfonic acid method	
Sewage .....	120
Water .....	69
Reduction method	
Sewage .....	119
Water .....	70
Nitric Acid Solution	
1:1 .....	249
1:9 .....	249
1:100 .....	249
1N .....	249
0.01N .....	249
Nitrite Nitrogen	
Sewage .....	121
Water	
Alpha-naphthylamine reagent .....	72
Standard solution .....	72
Sulfanilic acid reagent .....	72
Nitrogen	
Sewage	
Ammonia .....	115
Kjeldahl .....	119
Nitrate .....	119, 120
Nitrite .....	121
Organic .....	118
Sludge	
Ammonia .....	160
Organic .....	160
Total .....	159

Water .....	64-72
Albuminoid .....	68
Ammonia .....	64
Nitrate .....	69
Nitrite .....	71
Organic (Kjeldahl) .....	68
Non-carbonate Hardness .....	27
Non-incrustants in Water .....	9
<i>Non-standard Methods</i>	
Activity of activated sludge .....	219
British practice .....	233
Agar count .....	237
Classification of water .....	241
Coliform count .....	238
Interpretation of results .....	237
Presumptive coliform test .....	243
Rationale .....	234
Type of examination .....	236
Broths, selective for primary or parallel planting .....	226
Chromium in water .....	209
Differentiation—coliform groups .....	228
Broths for .....	226
Culture purification .....	228
Eijkman test .....	231
Indole test .....	230
Methyl red test .....	230
Reaction classification .....	239
Selective agar media for .....	227
Slow lactose fermenters .....	230
Sodium citrate test .....	231
Voges-Proskauer test .....	231
Grease in sludge	
Coagulated grease .....	223
Wet extraction .....	221
Lead (dithiozone method) .....	214
Organic carbon in sewage .....	224
Oxygen demand of activated sludge .....	219
pH buffers and indicators .....	211
Buffers .....	212
Indicators .....	213
pH of sewage and sludge colorimetrically .....	224
Phenols	
Polluted streams .....	218
Water .....	216
Selective agar media for differentiation in coliform groups .....	227
Selenium in water .....	210
Volatile acids in sludge .....	218
Normal Carbonate .....	8
Number of Organisms (Microscopic Exam.) .....	176
Nutrient Agar .....	186
Observations (Microscopic Exam.) .....	173
Ocular Micrometer .....	165



Odor	
Characteristics	19
Cold	17
Free water	16
Hot	17
Osmoscope	16
Parallel threshold chart	18
Precautions	16
Procedure for	17, 18
Quality	16
Reagents and apparatus	16
Sewage	115
Threshold odor concentration	18
Threshold odor number	17
Type of	19
Oil	42
Evaporation and extraction method	42
Wet extraction method	43
Organic Carbon in Sewage	224
Organic Nitrogen	
Kjeldahl method	68
Sewage	118
Water	68
Ortho-phenanthroline Method for Iron	52
Orthophosphate	79
Amino-naphthol sulfuric acid reagent for	80
Ammonium molybdate reagent for	79
Colorimetric method for	79
Amino-naphthol sulfuric acid	79, 80
Stannous chloride	80
Gravimetric method for	79
Standard solution of	81
Ortho-tolidine	
Arsenite method for residual chlorine	100
Flash test	100
Method for residual chlorine	
Sewage	147
Water	93
Purity	92
Reagent	97
Osmoscope for Odor Determination	16
Outline of Procedure for Dissolved Oxygen	135
Oxygen Consumed	122
Oxygen Demand of Activated Sludge	219
Palmitate Determination of Hardness	23
Parallel Planting, Broths for	226
Parallel Threshold Chart for Odors	18
Parts per Million to Equivalents per Million	7
Peptone (Bact. Exam.)	184
Perchloric Acid	
Method for silica	44
Reagent for potassium	62

Periodate Method for Manganese .....	56
Permanent Standards	
Ammonia .....	67
Chlorine, residual .....	95
Color .....	14
Iron .....	54, 55
Manganese .....	56, 57
Nitrite nitrogen .....	72
Silica .....	45
Turbidity .....	12, 13
Permanganate Oxygen Consumed .....	122
Permissible Variations in Media .....	188
Permutit, Folin's Ammonia	
Persulfate Method for Manganese .....	57
Petri Dishes .....	183
Phenanthroline Method for Iron .....	52
Phenoldisulfonic Acid Method for Nitrates .....	69
Phenolphthalein	
Alkalinity .....	9, 32
Indicator solution .....	250
Phenol Red Indicator Solution .....	250
Phenols	
Polluted streams .....	218
Water .....	216
Phosphate (see ortho-, meta-, and pyro-)	
Phosphates in Sulfate Determination .....	85
Phosphoric Acid Solution—0.1N .....	250
Photoelectric Filter Photometer .....	5
(See individual colorimetric determinations)	
Photoelectric Spectrophotometer .....	5
(See individual colorimetric determinations)	
Photometric Apparatus .....	5
pH Value .....	28
Buffers .....	211
Colorimetric .....	30
Electrometric .....	28
Indicators .....	30, 213
Relation to H-ion concentration .....	29
Sewage .....	147, 224
Sludge .....	158
Physical Tests	
Sewage .....	115
Sludge .....	157
Water .....	10-19
Pipettes (Bact. Exam.) .....	183
Plating (Bact. Exam.) .....	191
Platinum-Cobalt Standards	
For ammonia nitrogen .....	67
For color .....	14
For iron .....	54
Polluted Streams and Trade Wastes .....	113
Potassium .....	62
Colorimetric method for .....	64
Perchlorate .....	64

Gravimetric method for .....	62
Perechloric acid reagent for .....	63
Sodium cobaltinitrite reagent for .....	64
Potassium Chloroplatinate and Cobaltous Chloride	
For ammonia nitrogen standards .....	67
For color standards .....	14
For iron standards .....	54
Potassium Ferrocyanide Reagent for Zinc .....	58
Potassium Hydroxide Solution	
12 N .....	250
0.1341 N .....	250
Potassium Palmitate Solution for Hardness .....	23
Potassium Permanganate Solution	
0.2 N .....	250
0.1 N .....	250
Precautions for Odor Determination .....	16
Precision of Coliform Tests .....	206
Preparation of Media .....	184
Presumptive Test for Coliform .....	193, 194, 197
Primary or Parallel Planting, Broths for .....	226
Procedure (See Each Section)	
Biochemical oxygen demand	
Chlorinated sewage .....	142
Unchlorinated sewage .....	140
Protection of Chlorine Standards .....	97
Purity of Ortho-tolidine .....	92
Pyrophosphate	
Difference method for .....	82
Manganous chloride method for .....	81
Qualitative Samples (Microscopic Examination) .....	177
Quality of Odors .....	16
Quantitative Samples (Microscopic Examination) .....	177
Quantity of Water for Analysis .....	1
Quantity Units .....	114
Railroad Supply Water Determinations .....	10
Ratio of Ortho-tolidine to Chlorine .....	94
Reaction	
Culture media .....	184
Sludges and muds	
Acidity .....	158
Alkalinity .....	158
pH .....	158
Reaction Classification—Coliform Group .....	239
Reagent Grade Chemicals .....	246
Standardization samples .....	248
Reagents .....	5
(See individual determinations)	
Reagents, Common Laboratory .....	248
Acetic acid soln.—50% .....	248
Ammonium hydroxide soln.	
1:1 .....	248



*Reagents (cont.)*

1: 4 .....	248
1: 5 .....	248
1: 100 .....	248
Arsenous acid soln. 0.1 N, approx. ....	248
Brom cresol green indicator soln. ....	248
Ethyl alcohol soln.—1: 1 .....	248
Hydrochloric acid soln.	
1: 1 .....	249
1: 8 .....	249
1: 9 .....	249
1: 11 .....	249
1: 23 .....	249
1: 50 .....	249
6 N .....	249
5 N .....	249
0.248 N .....	249
0.1 N .....	249
0.02 N .....	249
Iodine soln.—0.025 N .....	249
Methyl orange indicator soln. ....	249
Methyl red indicator soln. ....	249
Nitric acid soln	
1: 1 .....	249
1: 9 .....	249
1: 100 .....	249
1 N .....	249
0.01 N .....	249
Phenolphthalein indicator soln. ....	250
Phenol red indicator soln. ....	250
Phosphoric acid soln.—0.1 N .....	250
Potassium hydroxide soln.	
12 N .....	250
0.1341 N .....	250
Potassium permanganate soln.	
0.2 N .....	250
0.1 N .....	250
Sodium carbonate soln.—0.02 N .....	250
Sodium hydroxide soln.	
2 N .....	250
1 N, carbonate free .....	250
0.5 N, carbonate free .....	250
0.025 N, approx. ....	251
0.1 N .....	251
0.0231 N .....	251
0.02 N .....	251
Sodium thiosulfate std. soln. 0.025 N .....	251
Starch indicator soln. ....	251
Sulfuric acid soln.	
1: 1 .....	251
1: 3 .....	251
1: 6 .....	251
1: 8 .....	251
1: 35 .....	251

*Reagents (cont.)*

2 N .....	251
1 N .....	251
0.1 N .....	252
0.02 N .....	252

*Recording Results*

Chemical .....	5
Color .....	14
Microscopic .....	172-180
Odor .....	18, 19
Turbidity .....	13

*Reduction Method for Nitrates*

Sewage .....	119
Water .....	70

*Relation of Methyl Orange to Phenolphthalein Alkalinity* ..... 9*Relation of pH, CO<sub>2</sub>, CO<sub>3</sub><sup>-</sup>, HCO<sub>3</sub><sup>-</sup>* ..... 35, 36*Relation of pH to H-ion Concentration* ..... 29*Relative Stability* ..... 144*Reporting Results*

(See "Expression of Results")

*Bacteriological* ..... 207*Chemical* ..... 5*Microscopic**Calculation* ..... 174*Cubic standard units* ..... 174*Number of organisms* ..... 176*Forms* ..... 172, 173*Observations* ..... 173*Sewage sludge* ..... 180*Representative Samples (Water)* ..... 2*Residual Chlorine**Sewage* ..... 147*Field test* ..... 150*Ortho-tolidine method* ..... 150*Spot plate test* ..... 150*Starch iodide method* ..... 148*Water* ..... 92*Discussion* ..... 92*Application of methods* ..... 92*Concentration of acid in O-T. reagent* ..... 92*Contact (reaction) time* ..... 92*Flash test* ..... 93*Iodometric method* ..... 93*Modified Scott standards* ..... 92*Ortho-tolidine arsenite test* ..... 93*Purity of ortho-tolidine* ..... 92*Temperature* ..... 92*Time limit on reagent* ..... 92*Zero chlorine demand water* ..... 92*Drop dilution method for* ..... 102*Contact time* ..... 102*Iodometric method for* ..... 98*Preparation of sample* ..... 100*Preparation for titration* ..... 100

*Residual Chlorine* (cont.)

## Water (cont.)

Reagents for .....	99
Standardization of chlorine solution .....	98
Starch solution .....	99
Titration .....	100
Volume of sample in .....	99
Ortho-tolidine arsenite method (OTA) .....	100
Determination of Results .....	101
Combined available chlorine .....	102
Free available chlorine .....	102
Interfering colors .....	102
Total residual chlorine .....	102
Procedure .....	101
Ortho-tolidine flash test .....	100
Laux flash test .....	100
Ortho-tolidine method for .....	93
Interfering substances .....	93
Light sources for .....	94
Procedure .....	97
Compensation for interference .....	98
Temperature effect .....	98
Ratio of ortho-tolidine to chlorine .....	94
Reagents .....	97
Ortho-tolidine .....	97
Standards, chlorine .....	94-97
Permanent .....	95
Buffer solutions .....	95
Protection of .....	97
Scott's (modified) formulas .....	97
Temporary .....	94
Chlorine solution for .....	95
Preparation of .....	95
Titration of chlorine solution .....	95
Zero chlorine demand water .....	94
Residue or Solids in Sewage .....	145
Residue (Water) .....	20
By calculation .....	22
By electrolytic conductivity .....	21
Dissolved .....	20
In condensed steam .....	22
On evaporation .....	20
Suspended .....	21
Total and total fixed .....	20
Results	
(See "Expression of Results")	
(See "Reporting Results")	
Rideal-Stewart Modification for D.O. ....	130
Sample	
Bacteriological .....	190
Bottles for bacteriological examination .....	183
Collection	
Bacteriological examination .....	190



Boiler water examination .....	2, 3
Chemical examination .....	1
Dissolved oxygen .....	124
Microscopic examination .....	162
Sewage .....	113
Sludges and muds .....	156
Collection devices	
Boiler water cooling coil .....	2, 3
Dissolved oxygen .....	125
Microscopic examination .....	162
Concentrating devices—microscopic exam. ....	163
Quantity required .....	1, 113
Representative .....	1, 113
Sewage .....	113
Preservation .....	113
Representative .....	113
Sludge and mud .....	156
Sulfide .....	153
Swimming pool .....	208
Time interval between collection and analysis ..	1
Water .....	1
Sampler	
Boiler water .....	2, 3
Dissolved oxygen .....	125
Methane .....	110
Microscopic examination .....	162
Sanchis' Method for Fluoride .....	76
Scott Modification for Fluoride Method .....	76
Scott Standards (Modified) for Residual Chlorine ..	92, 97
Sedgwick-Rafter Funnel Filter .....	163
Seeding for B.O.D. Determination .....	140
Selection of	
Method for dissolved oxygen .....	126
Tests for coliform bacteria .....	199
Selective Agar Media for Coliform Group .....	227
Selective Broths for Primary or Parallel Planting ..	226
Selenium .....	210
Settleable Solids	
By volume .....	146
By weight .....	146
Sewage .....	113
Acidity .....	146
Alkalinity .....	147
Ammonia nitrogen .....	115
Ash .....	145
Biochemical oxygen demand .....	139
Calculation of .....	141-142
Dilution method for	
Sewage .....	139
Sludges and muds .....	143
Procedure for	
Chlorinated sewage .....	142
Unchlorinated sewage .....	140
Seeding .....	140

*Sewage (cont.)*

Standard dilution water .....	140
Carbon, Organic .....	224
Chloride .....	147
Chlorine demand .....	150
Color .....	115
Dissolved oxygen .....	124
Activated sludge .....	134, 136
Alkaline hypochlorite method .....	132, 136
Alsterberg method .....	129, 135
Alum flocculation method .....	134, 138
Calculation of results .....	138
Outline of procedure for .....	135
Rideal-Stewart modification for .....	130
Sampler for .....	125
Samples for .....	124
Selection of method for .....	126
Sodium azide method for .....	129, 135
Standardization of thiosulfate for .....	126
Winkler method .....	127, 135
Dissolved solids .....	145
Expression of chemical results .....	114
Fixed solids .....	145
Suspended .....	145
Total .....	145
Grease .....	
Sewage .....	155
Sludge .....	221, 223
Kjeldahl nitrogen .....	119
Nitrate nitrogen .....	119
Phenoldisulfonic acid method for .....	120
Reduction method for .....	119
Nitrite nitrogen .....	121
Odor .....	115
Organic carbon .....	224
Organic nitrogen .....	118
Oxygen consumed .....	122
Oxygen demand of activated sludge .....	219
pH value .....	147
Buffers .....	211
Colorimetrically .....	224
Indicators .....	213
Phenols .....	218
Physical examination of .....	115
Quantity units .....	114
Relative stability .....	144
Residual chlorine .....	147
Residue (see solids) .....	145
Samples .....	113
Collection of .....	113
Preservation of .....	113
Representative .....	113
Settleable solids .....	
By volume .....	146

*Sewage (cont.)*

By weight .....	146
Sludge and muds .....	156
Activated .....	158
Grease .....	160
Microscopic examination .....	160
Moisture .....	159
Nitrogen .....	159-160
Physical tests .....	157
Reaction .....	158
Sample collection .....	156
Solids .....	159
Specific gravity .....	157
Solids .....	
Ash .....	145
Dissolved .....	146
Fixed .....	
Suspended .....	145
Total .....	145
On evaporation .....	
Fixed .....	145
Total .....	145
Volatile .....	145
Settleable .....	
By volume .....	146
By weight .....	146
Suspended .....	145
Fixed .....	145
Total .....	145
Volatile .....	145
Total .....	145
Volatile .....	
Suspended .....	145
Total .....	145
Sulfides .....	152
Colorimetric method .....	154
Dissolved .....	155
H <sub>2</sub> S .....	155
Limitations .....	155
Total .....	155
Titration method .....	152
Dissolved .....	153
H <sub>2</sub> S .....	153
Sampling .....	153
Total .....	153
Suspended solids .....	
Fixed .....	145
Total .....	145
Volatile .....	145
Temperature .....	115
Total Kjeldahl nitrogen .....	119
Total solids .....	145
Turbidity .....	115



*Sewage (cont.)*

Volatile solids	
Suspended	145
Total	145
Sieves (Microscopic Exam.)	177
Silica	43
Colorimetric	44
Gravimetric	43
Hydrochloric acid method	44
Perechloric acid method	44
In condensate	45
Molybdate method	44
Sampling	43, 44
Standards	45
Slow Lactose Fermenters	230

*Sludge and Muds*

Activated sludge	
Density index (S.D.I.)	158
Oxygen demand and activity	219
Settleability	157
Suspended solids	157
Volume index (S.V.I.)	158
Biochemical oxygen demand	143
Grease	150
Coagulated	223
Wet extraction	221
Microscopic examination	177
Apparatus	177
Collection of samples	178
Concentration of samples	178
Definition and scope	177
Enumeration of organisms	179
Reporting results	180
Moisture (see solids)	159
Nitrogen	
Ammonia	160
Organic	160
Total	159
Physical tests	157
Reaction	158
Acidity	158
Alkalinity	158
pH	159
Sample collection	156
Solids	159
Moisture	159
Total	159
Volatile	159
Specific gravity	157
Volatile acids	218
Soap Hardness	25
Soap Solution, Standard, for Hardness	25
Soda Reagent for Hardness	25

Sodium Azide Modification for D.O. ....	129, 135
Sodium Carbonate Solution—0.02 N .....	250
Sodium	
Gravimetric method for .....	62
Zinc uranyl acetate reagent for .....	62
Sodium Citrate Test (Bact. Exam.) .....	231
Sodium Cobaltinitrite Reagent for Potassium .....	64
Sodium Hydroxide Solution	
2 N .....	250
1 N, carbonate free .....	250
0.5 N, carbonate free .....	250
0.025 N, approx. ....	251
0.1 N .....	251
0.0231 N .....	251
0.02 N .....	251
Sodium Paraperiodate Reagent for Manganese .....	57
Sodium Sulfite Reduction of Molybdate for Silica .....	46
Sodium Thiosulfate for D.O. ....	126
Sodium Thiosulfate Solution—0.025 N .....	251
Solids (See also "Residue")	
Sewage .....	145
Ash .....	145
Dissolved .....	146
Fixed	
Suspended .....	145
Total .....	145
On evaporation	
Fixed .....	145
Total .....	145
Volatile .....	145
Settleable	
By volume .....	146
By weight .....	146
Suspended	
Fixed .....	145
Total .....	145
Volatile .....	145
Total .....	145
Volatile	
Suspended .....	145
Total .....	145
Sludge	
Moisture .....	159
Total .....	159
Volatile .....	159
Water .....	20-22
Solubility of Oxygen	
In fresh water .....	137
In sea water .....	137
Specialized Apparatus .....	5
Specific Gravity (Sludge) .....	157
Spot Plate Test (Residual Chlorine) .....	150
Stage Micrometer .....	166

Standard Dilution Water for B.O.D. ....	140
Standardization of Chlorine Solution .....	98
Standards	
(See also individual determinations)	
Ammonia nitrogen .....	67
Chlorine, residual .....	95
Color .....	14
Iron .....	54
Manganese .....	56
Nitrite nitrogen .....	72
Silica .....	45
Turbidity .....	12
Standard Tests for Coliform Bacteria .....	193
Stannous Chloride Reagent for Orthophosphate .....	80
Starch Indicator Solution .....	99, 109, 251
Starch—Iodide Method for Residual Chlorine	
Sewage .....	148
Water .....	93, 98
Storage of Media .....	189
Strip Count (Microscopic Exam.) .....	171
Strontium Chloride, Method for .....	40
Sugars (Bact. Exam.) .....	184
Sulfanilic Acid Reagent for Nitrites .....	72
Sulfate .....	83
Benzidine hydrochloride reagent for .....	84
Benzidine method for .....	84
Gravimetric method for .....	83
Tetrahydroxyquinone method for .....	85
With phosphate absent .....	85
With phosphate present .....	85
Sulfide Method for Lead .....	49
Sulfides	
Sewage .....	152
Colorimetric method .....	154
Dissolved .....	155
H <sub>2</sub> S .....	155
Limitations .....	155
Total .....	155
Titration method .....	152
Dissolved .....	153
H <sub>2</sub> S .....	153
Sampling .....	153
Total .....	153
Water	
Colorimetric method for .....	87
Dissolved .....	87
Evolution method for .....	87
Total .....	87
Sulfite, Volumetric Method for .....	86
Sulfuric Acid Solution	
1: 1 .....	251
1: 3 .....	251
1: 6 .....	251



1: 8 .....	251
1: 35 .....	251
2 N .....	251
1 N .....	251
0.1 N .....	252
0.02 N .....	252
Survey Count (Microscopic Exam.) .....	173
Suspended Solids .....	
Sewage .....	145
Water .....	21
Swimming Pool Control .....	207
Bacterial count .....	208
Coliform group .....	208
Preparation of sample bottle .....	208
Sample collection .....	208
Tannin and Lignin .....	91
Tyrosine reagent for .....	91
Temperature .....	
In residual chlorine tests .....	92, 98
Sewage .....	115
Water .....	10
Temporary Standards for Residual Chlorine Determination .....	94
Tetrahydroxyquinone Method for Sulfates .....	85
Thiocyanate Method for Iron .....	53
Threshold Odor .....	
Concentration .....	18
Number .....	17
Time Interval Between Collection and Analysis .....	1
Time Limit on Ortho-tolidine Reagent .....	92
Titan Yellow Reagent for Magnesium .....	61
Titration of Free Carbon Dioxide .....	34
Total .....	
Acidity .....	31
Alkalinity .....	9, 31
Carbon dioxide .....	32
Chromium .....	56
Hardness .....	23, 25, 27
Iron .....	52, 53
Residual chlorine .....	102
Residue .....	20, 21, 145
Solids .....	20, 21, 145
Sulfides .....	87, 152
Trade Wastes .....	113
Tryptone Glucose Extract Agar .....	186
Tubes .....	
Fermentation .....	183
Nessler .....	4
Turbidimeter .....	
Baylis .....	13
Candle .....	11
Jackson .....	11
St. Louis .....	13

Turbidity .....	10
Definitions .....	10
Measurements	
Above 100 .....	12
Between 5 and 100 .....	12
Less than 5 .....	13
Standard .....	10
Procedure for .....	12, 13
Recording readings .....	13
Sewage .....	115
Standards .....	12
Types of Odors .....	19
Tyrosine Reagent for Tannin and Lignin .....	91
Unchlorinated Sewage, B.O.D. of .....	140
Uncombined Carbon Dioxide	
By calculation .....	34
By titration .....	34
Units of Chemical Results .....	9
U. S. Bureau of Standards .....	248
Standardization samples .....	248
Variations in Media, Permissible .....	188
Violet Red Agar .....	228
Voges-Proskauer Test (Bact. Exam.) .....	231
Volatile Acids .....	218
Volatile Solids	
Sewage .....	145
Volhard Method for Chloride .....	73
Volumetric	
Determinations	
Acidity .....	31
Alkalinity .....	31
Calcium .....	59
Sulfides .....	87, 152
Sulfite .....	86
Glassware .....	5
Standard unit (microscopic exam.) .....	174
<i>Water</i> .....	1
Acidity in .....	31
Albuminoid nitrogen in .....	68
Alkalinity in .....	31
Aluminum-free .....	50
Aluminum in .....	50
Ammonia-free .....	65
Ammonia nitrogen in .....	64
Arsenic in .....	46
Bacteriological examination	
Agar, selective .....	226
Apparatus .....	183
British practice .....	233
Broths, selective .....	227
Coliform group .....	193, 228

*Water (cont.)*

Collection of samples .....	190
Counting .....	192
Culture media .....	184
Dilutions .....	191
Incubation .....	192
Materials .....	184
Plating .....	191
Swimming pool control .....	207
Bicarbonate ion in .....	37
Boiler .....	10
Boiler feed .....	10
Boron in .....	87
Calcium in .....	59
Carbonate ion in .....	38
Carbon dioxide in .....	32
Chloride in .....	73
Chlorine demand-free .....	92, 94
Chlorine demand of .....	103
Chlorine reagent .....	151
Chlorine, residual, in .....	92
Chromium in .....	55, 209
Collection of samples .....	1
Color .....	14
Copper in .....	47
Cyanide in .....	90
Determinations .....	10
Dissolved oxygen in .....	106
Distilled .....	4
Drinking quality .....	202
Examination of .....	1
Bacteriological .....	183
Chemical .....	20-112
Introduction to .....	1
Microscopic .....	162
Physical .....	10-19
Fluoride in .....	76
Hydrogen sulfide in .....	110
Hydroxide ion in .....	40
Iodide in .....	74
Iron-free distilled .....	52
Iron in .....	51
Lead in .....	49, 214
Lignin in .....	91
Magnesium-free .....	61
Magnesium in .....	60
Manganese in .....	56
Metaphosphate in .....	82
Methane in .....	111
Microscopic examination .....	162
Apparatus .....	162
Collection of samples .....	166
Concentration of samples .....	167



*Water (cont.)*

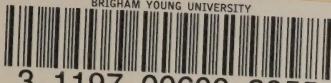
Definitions and scope .....	162
Enumeration .....	170
Reporting results .....	173
Nitrate nitrogen in .....	70
Nitrite nitrogen in .....	71
Nitrogen in .....	64-72
Oil in .....	42
Orthophosphate in .....	79
Organic nitrogen in .....	68
Odor determination of .....	16
Odor-free .....	16
Other than drinking quality .....	203
Phenol in .....	216
pH value of .....	28
Buffers .....	212
Indicators .....	213
Pyrophosphate in .....	81
Quantity required for analysis .....	1
Railroad supply .....	10
Residual chlorine in .....	92
Residue in .....	20
Selenium in .....	210
Silica in .....	44
Sodium in .....	62
Solids in .....	20
Standard dilution for B.O.D. ....	140
Subject to U.S.P.H.S. standards .....	202
Sulfate in .....	83
Sulfides in .....	87
Sulfite in .....	86
Tannin in .....	91
Temperature .....	10
Turbidity .....	10
Zinc in .....	58
Wet Extraction for Grease .....	221
Wet Extraction of Oil .....	43
Winkler Method for D.O. ....	127, 135
Zeolite Effluent Hardness .....	25
Zero Chlorine Demand Water .....	92, 94
Zinc	
Nephelometric method for .....	58
Potassium ferrocyanide reagent for .....	58
Standard solution of .....	58
Zinc Uranyl Acetate Reagent for Sodium .....	62
Zirconium Alizarin Reagent for Fluoride .....	76

STANDARD METHODS FOR THE EXAMINATION OF WATER AND SEWAGE

NINTH  
EDITION  
1946







3 1197 00600 3955

*Samuel J. Brown*

## DATE DUE

JUL 23

JUL 28 1987

JAN 20 1993

DEC 15 1992

JAN 24 1993

DEC 29 1992

DEC 17 1992

DEC 26 1996



